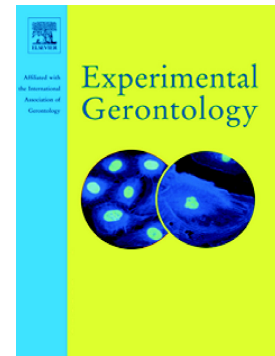


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## **Role of D-galactose-induced brain aging and its potential used for therapeutic interventions**

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**Abstract**

Aging is a phenomenon that all living organisms inevitably face. Every year, 9.9 million people, globally, suffer from dementia, an indicator of the aging brain. Brain aging is significantly associated with mitochondrial dysfunction. This is characterized by a decrease in the activity of respiratory chain enzymes and ATP production, and increased free radical generation, mitochondrial deoxyribonucleic acid (DNA) mutations, and impaired mitochondrial structures. To get a better understanding of aging and to prevent its effects on many organs, chronic systemic administration of D-galactose was used to artificially create brain senescence in animal models and established to be beneficial for studies of anti-aging therapeutic interventions. Several studies have shown that D-galactose-induced brain aging which does so not only by causing mitochondrial dysfunction, but also by increasing oxidative stress, inflammation, and apoptosis, as well as lowering brain-derived neurotrophic factors. All of these defects finally lead to cognitive decline. Various therapeutic approaches which act on mitochondria and cognition were evaluated to assess their effectiveness in the battle to reverse brain aging. The aim of this article is to comprehensively summarize and discuss the underlying mechanisms involved in D-galactose-induced brain aging, particularly as regards alterations in brain mitochondria and cognitive function. In addition, the aim was to summarize the different therapeutic approaches which have been utilized to address D-galactose-induced brain aging.

**Keywords:** D-galactose, brain aging, mitochondria, cognitive function, therapeutic

## Introduction

The world's population is becoming increasingly aged. According to the United Nations' World Population Prospects, the percentage of people aged 60 or over was reported to be 12.3% in 2015 and it is projected to rise to 21.5% of the global population by 2050 (Sander et al., 2015). Due to the falling fertility rates and remarkable increases in life expectancy around the world, age-related problems have come to the forefront of current attention (Sander et al., 2015). Dementia or cognitive impairment is the major cause of disability and dependency among elderly people worldwide, which is why WHO have reported dementia as being a public health priority (Wortmann, 2012). It is forecasted that the number of people affected by dementia will double every 20 years and it is acknowledged that no one is immune to brain aging disorders (Gillum et al., 2011).

Mitochondrial dysfunction plays an important role in brain aging or brain senescence, and several aspects of age-associated neurodegeneration (Ames, 2004; Grimm and Eckert, 2017; Sanz and Stefanatos, 2008; Schriener et al., 2005; Wright et al., 2004). Increased production of reactive oxygen species (ROS) following mitochondrial dysfunction induces brain oxidative stress via a vicious cycle of ROS-induced ROS release in mitochondria, and this possibly leads to brain aging or brain senescence (Pak et al., 2003). The senescence of the brain finally leads to cognitive impairment, which is the primary symptom of several neurodegenerative diseases in elderly people. To investigate the underlying mechanisms of brain aging, several animal models have been used. The D-galactose accelerated brain-aging process in animal models is the most common model used for investigating the brain aging process (Haider et al., 2015; Lu et al., 2007; Lu et al., 2010b; Xian et al., 2014; Zhang et al., 2011; Zhu et al., 2014). The administration of D-galactose into animals can induce aspects of brain aging similar in many ways to human brain aging, including memory deficit, neuronal degeneration and apoptosis, raised oxidative stress, decreased ATP production, increased

mitochondrial DNA mutation, impaired mitochondrial structure and control abnormal gene expression in the brain (Banji et al., 2014; Kumar et al., 2009; Lei et al., 2008; Prakash and Kumar, 2013; Ullah et al., 2015).

D-galactose is an aldohexose that occurs naturally in the body, including in the brain (Nagy and Pohl, 2015). However, it is known that when an exogenous dose of D-galactose is given beyond normal concentration, this can induce aging effects in several organs by increasing oxidative stress, apoptosis and inflammation (Qu et al., 2016; Rehman et al., 2017; Ullah et al., 2015). Among the various organs in the body, the brain is the organ most vulnerable to oxidative stress due to its high metabolic activity, high lipid content and limited antioxidant defense mechanisms (Çakatay, 2010). In addition, several studies have described that brain aging is attributed to mitochondrial functions (Banji et al., 2014; Chen et al., 2011; Du et al., 2012; Du et al., 2015; Kumar et al., 2009; Long et al., 2007; Prakash and Kumar, 2013; Zeng et al., 2014). The underlying mechanisms of D-galactose-induced brain aging have yet to be discovered. The aim of this article is to comprehensively summarize and discuss the underlying mechanism of D-galactose-induced brain aging, particularly via alterations in brain mitochondria and cognitive function as well as to summarize the different therapeutic approaches on D-galactose-induced brain aging. The article also discusses the findings that elucidate mitochondrial DNA mutations, respiratory chain enzymes, antioxidant ability and structural changes in the aging brain. It additionally outlines mitochondria-targeted therapies in D-galactose-induced brain senescence models.

### **1. D-galactose Metabolism**

D-galactose is a reducing sugar and it occurs in many foods such as honey, beets, cheese, yoghurt, butter, milk, kiwi fruit, soy sauce, plums, dry figs, cherries, and celery (Acosta and Gross, 1995). When D-galactose rich food is eaten, the sugar reaches the intestinal lumen and it is transported by sodium-dependent glucose cotransporters type 1

(SGLT-1) into the cells and leaves the cells by glucose transport type 2 (GLUT-2), then enters the blood stream (Bjelakovic et al., 2011). Normally, two enzymes, galactokinase and uridyl transferase, metabolize D-galactose into glucose, which enters the glycolysis pathway or is stored as glycogen in liver, muscle and adipose tissue (Coelho et al., 2015). The mediation of the uptake of D-galactose into the brain through blood brain barrier is by glucose transport type 1 (GLUT-1) (Cura and Carruthers, 2012). The normal concentration of D-galactose in the blood is less than 10 mg/dL (Berry, 1993). For a healthy adult the maximal recommended daily dose is 50 g of galactose and most of it can be eliminated from the body within about 8 hours after ingestion (Morava, 2014).

However, oversupply of D-galactose can give rise to the generation of ROS causing mitochondrial dysfunction, oxidative stress, inflammation and apoptosis in neuronal cells (Kumar et al., 2009; Prakash and Kumar, 2013; Qu et al., 2016; Rehman et al., 2017; Ullah et al., 2015). Therefore, the use of long-term injections of D-galactose is a well-known method in the study of aging, as indicated by increased aging markers such as advanced glycation end products (AGE), receptors for advanced glycation end product (RAGE), aldose reductase (AR), sorbitol dehydrogenase (SDH), telomere length shortening, telomerase activity, beta-site amyloid precursor protein cleaving enzyme 1 (BACE-1), amyloid beta protein ( $A\beta_{1-42}$ ), senescence-associated genes ( $p^{16}$ ,  $p^{21}$ ,  $p^{53}$ ,  $p^{19Arf}$ ,  $p^{21Cip1/Waf1}$ ) and senescence-associated beta-galactosidase (SA- $\beta$ -gal) staining. All of these findings are summarized in Table 1.

In addition to the chemical changes several previous studies have demonstrated that addition of a chronic supply of D-galactose causes a deterioration in cognitive function that is correlated to symptoms of brain aging and thus, is used as the model of an accelerated aging brain in rodents (Haider et al., 2015; Lu et al., 2007; Lu et al., 2010b; Xian et al., 2014; Zhang et al., 2011; Zhu et al., 2014). The important underlying mechanisms of brain aging are related to increased oxidative stress and mitochondrial dysfunction. The effect of D-

galactose-induced brain aging on oxidative stress and mitochondrial dysfunction are crucial to the understanding of the aging process and are presented and discussed in the following paragraph.

## **2. The effects of D-galactose-induced mitochondrial dysfunction and oxidative stress in the brain**

There is a growing body of evidence which indicates that the mechanism of D-galactose-induced oxidative stress is occurring at a sub-cellular level, specifically in the brain mitochondria (Banji et al., 2014; Kumar et al., 2009; Prakash and Kumar, 2013; Zhang et al., 2010). When there is increase in D-galactose concentration, it is oxidized by galactose oxidase to form hydrogen peroxide ( $H_2O_2$ ), leading to decrease in superoxide dismutase (SOD) (Hsieh et al., 2009). Increased  $H_2O_2$  reacts with a reduced form of iron (Fe) to form hydroxide ions ( $OH^-$ ). The  $H_2O_2$  and  $OH^-$  are both types of reactive oxygen species (ROS) and along with others can cause lipid peroxidation in the cell membranes and impair redox homeostasis, leading to neuronal damage (Hsieh et al., 2009). In addition, D-galactose reacts with amines to form an unstable compound (called Schiff's base product) which undergoes several reactions over a period of days to form a more stable compound known as the Amadori product (Ansari and Dash, 2013; Golubev et al., 2017). This Amadori product converts irreversibly to a compound known as an advanced glycation end product (AGE) over months/years (Hsieh et al., 2009). When AGE binds with its receptor RAGE, an increase in nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and ROS production occurs, resulting in neuronal damage and cognitive dysfunction (Hsieh et al., 2009). Additionally, a high level of D-galactose is reduced by galactose reductase to form galactitol which results in osmotic stress and reduces the activity of the electron transport chain (ETC) in the mitochondria with resulting increased ROS production, finally causing mitochondrial dysfunction (Hsieh et al., 2009). Other oxidative stress markers in D-

galactose-induced brain aging assessed in those studies were malondialdehyde (MDA), nitrite level,  $\text{H}_2\text{O}_2$ , 8-oxoguanine,  $\text{p91}^{\text{phox}}$ ,  $\text{p22}^{\text{phox}}$ ,  $\text{p47}^{\text{phox}}$ ,  $\text{p67}^{\text{phox}}$  of NADPH oxidase (NOX) 2, total nitric oxide synthase (TNOS), inducible nitric oxide synthase (iNOS), ROS, protein carbonyl and AOPP.

As regards D-galactose reduced respiratory chain enzymes, four studies pointed out that all respiratory chain complexes enzymes such as NADH-co Q oxidoreductase (I), succinate-co Q oxidoreductase (II), co Q-cytochrome C oxidoreductase (III), cytochrome C oxidase (IV) were damaged as a result of D-galactose injection (Banji et al., 2014; Kumar et al., 2009; Prakash and Kumar, 2013; Zhang et al., 2010). However, Long and colleagues found that only succinate-co Q oxidoreductase activity was affected by D-galactose (Long et al., 2007). The differences in those findings might be due to differing methods being used to measure respiration enzyme activity. D-galactose not only reduced the activity of respiratory enzymes, but also the levels of tricarboxylic acid cycle enzymes (Banji et al., 2014). Chen and colleagues also showed that D-galactose decreased the antioxidant ability of mitochondria from the cerebral cortex via benzodiazepine receptors (Chen et al., 2008). These findings suggested that benzodiazepine receptors might be part of the mechanisms controlling mitochondrial respiration to protect against damage from ROS (Carayon et al., 1996).

In addition, several studies described that D-galactose induced the accumulation of brain mitochondrial DNA mutation (Chen et al., 2011; Du et al., 2012; Du et al., 2015; Zeng et al., 2014) through: 1) decreasing DNA-repairing enzymes (OGG1,  $\text{pol } \gamma$ ) (Chen et al., 2011), and 2) common deletion of mitochondrial DNA and impairing mitochondrial structures via the NOX-dependent pathway (Du et al., 2015).

Accumulating evidence indicates that D-galactose could induce brain aging by causing oxidative stress and mitochondrial dysfunction in different brain regions such as the

hippocampus, cerebral cortex, auditory cortex and ventral cochlear nucleus (as shown in Table 2). D-galactose-induced brain aging, as indicated by brain mitochondrial dysfunction and structural change in these studies was in the dose-independent manner, starting from 100mg/kg/day to 500mg/kg/day following 6-8 weeks of the administration (Banji et al., 2014; Chen et al., 2011; Du et al., 2012; Du et al., 2015; Kumar et al., 2009; Long et al., 2007; Prakash and Kumar, 2013; Zeng et al., 2014).

Interestingly, D-galactose also led to a decrease in the antioxidant enzymes such as glutathione, catalase, superoxide dismutase, glutathione-S-transferase activity, glutathione peroxidase and total antioxidant capacity. Therefore, the imbalance between reactive oxygen species and antioxidant activities in D-galactose-induced aging models leads to increased oxidative stress and mitochondrial dysfunction, which are significant in the aging process.

All of those studies suggest that D-galactose-induced mitochondrial dysfunction as indicated by a reduction in respiratory chain enzymes and antioxidant activity, go on to increase oxidative stress, mitochondria DNA mutations, the decline of ATP synthesis, mitochondrial membrane potential changes and cause impairment of mitochondrial structures. D-galactose not only caused mitochondrial dysfunction and oxidative stress, but also induced neuronal apoptosis. All of these findings are summarized in Table 2. The following paragraph will discuss the effect of D-galactose-induced apoptosis in the brain.

### **3. Effect of D-galactose-induced apoptosis in the brain**

D-galactose activates both extrinsic and intrinsic pathways of apoptosis (as shown in Table 2). The extrinsic ‘death receptor’ pathway directly activates effector caspases via JNK (c-Jun-N-terminal kinase) and converges with the intrinsic apoptotic pathway at the mitochondrion (Benn and Woolf, 2004). It was found that D-galactose activated p-JNK and enhanced the level of the cytochrome complex (cyt c) which stimulated the activation of caspase-3, caspases-9 and cleaved poly ADP ribose polymerase (PARP-1) (Ali et al., 2015).

In addition, D-galactose triggered the mitochondria to release cyt c, reduced anti-apoptotic Bcl2 expression level and increased apoptotic Bax. All of those events suggested that D-galactose promoted the apoptotic process (Qian et al., 2008). In addition to apoptosis, D-galactose promoted neuro-inflammation and neurodegeneration (Cui et al., 2006). The dosage from which D-galactose started inducing apoptosis was 100 mg- 500 mg/kg/day, duration was from 6 weeks to 9 weeks. (as shown in Table 2)

#### **4. Effect of D-galactose-induced brain inflammation**

Inflammatory markers used for monitoring in D-galactose-induced aging models are cyclooxygenase (COX-2), iNOS, NOS-2, tumor necrosis factor alpha (TNF- $\alpha$ ), interleukin (IL-1 $\beta$ ), IL-6, nuclear factor (NF- $\kappa$ B) thioredoxin-interacting protein (Txnip), p-NF- $\kappa$ Bp65, p-I $\kappa$ B $\alpha$ , p-IKK $\alpha$ , p-IKK $\beta$  as shown in Table 2. All of these studies suggested that D-galactose increased the inflammatory markers and induced neuro-inflammation via the activation of the transcription factor NF $\kappa$ -B through Ras and redox-sensitive signaling pathways, resulting in memory impairment. D-galactose started inducing inflammation at a dosage of 50mg-180mg/kg/day and duration was from 6 weeks to 60 days.

#### **5. Therapeutic approaches to brain mitochondrial aging induced by D-galactose**

There are 6 therapeutic studies which focused on the mitochondria in D-galactose-induced brain aging as shown in Table 3. The first one used R-alpha-lipoic acid which is an antioxidant abundant in yeast, liver, kidney, spinach, broccoli, and potatoes, to treat mitochondrial dysfunction (Long et al., 2007). R-alpha-lipoic acid increased state 3 respiration which represents succinate-linked respiration and respiratory control ratio (state 3/state 4), but did not increase the ADP/O ratio (Long et al., 2007). However other therapeutic treatments helped to improve the activity of all respiratory enzyme complexes in the mitochondria as summarized in Table 3. (Banji et al., 2014; Kumar et al., 2009; Prakash and Kumar, 2013; Zhang et al., 2010). The possible reason may be due to inadequate dosages

of R-alpha-lipoic acid. One of the studies stated that 100 mg/kg/day of R-alpha-lipoic acid is used for prevention but not for intervention, dosage should be increased up to 180mg/kg/day (Inman et al., 2013).

Carvidilol, pioglitazone, curcumin and hesperidin have an antioxidant effect. These drugs have been used as an intervention to reverse D-galactose-induced mitochondrial dysfunction in a dose dependent manner. The possible underlying mechanisms of these drugs may be by increasing antioxidant enzymes, oxidative phosphorylation, and cell viability in senescence models (Banji et al., 2014; Kumar et al., 2009; Prakash and Kumar, 2013). In addition, catalpol alleviated oxidative stress and improved D-galactose-induced mitochondrial dysfunction in a dose independent manner, as indicated by a decrease in oxidative stress (Zhang et al., 2010). Chen and colleagues also showed that dehydroepiandrosterone (DHEA) attenuated D-galactose-induced mitochondrial dysfunction by strengthening the antioxidant ability via mitochondrial peripheral benzodiazepine receptors (Chen et al., 2008).

## **6. Effect of D-galactose on cognitive function**

Numerous studies found that systemic long-term administration of D-galactose has been used extensively to mediate the deterioration of cognitive function correlated to symptoms of aging (Lu et al., 2010b; Rehman et al., 2017; Yu et al., 2015; Zhu et al., 2014). Dosage of D-galactose impairment of cognitive function started from 50 mg to 180 mg/kg/day within a duration of 6-9 weeks. The tests used to detect the cognitive functions which monitor the effect of D-galactose-induced aging models were the following tests: Morris water maze, open field, step-through passive avoidance task, step-down, elevated plus maze paradigm and Y-maze as summarized in Table 4. Cognitive impairment in the D-galactose model has been shown to be caused by mitochondrial dysfunction, oxidative stress,

apoptosis, inflammation and aging (Lu et al., 2010b; Rehman et al., 2017; Yu et al., 2015; Zhu et al., 2014).

It is known that the learning and memory deficit occurring in age-related neurodegenerative disorders is associated with cholinergic decline (Lu et al., 2010a). Therefore, the acetylcholinesterase (AChE) activity and acetylcholine (ACh) level were estimated after behavioral tests. According to previous studies, D-galactose exerted a significant increase in AChE activity and a decrease in ACh level compared with the controls (Kumar et al., 2009; Qu et al., 2016; Yang et al., 2016; Yu et al., 2015).

Three types of glial cells such as astrocytes, oligodendrocytes, and microglia are seen in the brain. The most abundant cells in the central nervous system are astrocytes (Ronaldson and Bendayan, 2008). Previous studies revealed that the activation of microglia cells and astrocytes plays pivotal roles in neurodegenerative disorders (Benner et al., 2004; Dauer and Przedborski, 2003; Lu et al., 2010a; Wu et al., 2002). Glial fibrillary acidic protein (GFAP) and CD11b are specific markers for activated microglia cells and astrocytes, respectively. When D-galactose was given, activated microglial cells and astrocytes in hippocampus, prefrontal cortex and whole brain were observed (Lu et al., 2010b; Rehman et al., 2017; Zhu et al., 2014).

Brain-derived neurotrophic factor (BDNF) is essential for neuronal proliferation, excitability, synaptic transmission and plasticity. In addition, BDNF plays a crucial role in supporting the survival and growth of sensory and motor neurons, all of which have major roles in cognitive function (Takeda et al., 2014). D-galactose caused BDNF deficit, subsequently leading to cognitive impairment (Chen et al., 2016).

These findings suggested that D-galactose impaired cognitive function via the mechanisms involved in the increase in oxidative stress, apoptosis, inflammation, and

neuromodulation, the activation of astrocytes, microglia, BDNF deficiency and the decrease in antioxidant enzymes. All of these findings are summarized in Table 4.

## **7. Therapeutic approaches addressing cognitive function in D-galactose-induced aging models**

Various pharmacological anti-aging agents have been used to reverse the D-galactose-induced cognitive impairment. Among them, the most frequently used interventions are potent antioxidants, including ferulic acid, carvidilol, pioglitazone, curcumin and hesperidin. Those antioxidants improved cognitive function in a dose-dependent manner. In addition, anthocyanin, ursolic acid and ginsenoside are antioxidants that have been shown to improve cognitive function in the D-galactose model (Lu et al., 2010b; Rehman et al., 2017; Zhu et al., 2014). The other therapeutic approaches to improve cognition with a dose-independent manner in the D-galactose model were fibroblast growth factor 21 (FGF21), salidroside, and tetrahydropalmatine (Banji et al., 2014; Gao et al., 2015; Kumar et al., 2009; Prakash and Kumar, 2013; Qu et al., 2016; Yang et al., 2016; Yu et al., 2015). The dose-independent manner may be due to limitations of their receptors in the brain, while the activity of antioxidants depends on dosage.

FGF21 acts on its receptors and prevents D-galactose-induced spatial learning and memory impairment by attenuating oxidative stress, inflammatory markers and renovating the activities of antioxidant enzymes and decreasing AGE formation, and TChE activity (Yu et al., 2015). While salidroside combines with numerous receptors and regulates several signaling pathways such as those for axonal guidance, glutamate reception, G-protein coupled reception, cAMP-mediation, endothelial nitric oxide synthase (eNOS), ephrin reception, and atherosclerosis signaling pathways, which all were important in the prevention of D-galactose-induced cognitive decline (Panossian et al., 2014). Tetrahydropalmatine is a dopamine receptor antagonist that can prevent D-galactose-induced spatial learning and

memory impairment through decreasing oxidative damage, dysfunction of the cholinergic system, inflammatory markers and activation of astrocytes (Qu et al., 2016). Hyperbaric oxygen therapy, also known as HBOT, is a medical treatment which delivers oxygen to patients inside a compressed air chamber (Chen et al., 2016; Lu et al., 2010b; Zhu et al., 2014). HBO treatment can protect against cognitive impairment and hippocampal senescence by retaining the hippocampal BDNF expression and levels of antioxidants, reducing inflammation and modulating the aging-related gene expression in the D-galactose-induced aging mouse (Chen et al., 2016). Caffeine is 1, 3, 7-trimethylxanthine and is well known as the world's most famous psychoactive drug. Many studies have proved that a daily intake of caffeine ameliorated cognitive decline in “non-demented” elderly men and women by the mechanism of reducing synaptic dysfunction, and the reduction in oxidative stress, apoptosis, neuro-inflammation and neurodegeneration (Ritchie et al., 2007; van Gelder et al., 2007). Transcranial low-level laser therapy increased active mitochondrial levels, membrane potential, ATP production and abrogated oxidative stress and apoptosis, leading to a reversal in cognitive impairment in D-galactose-induced aging mice (Salehpour et al., 2017). All of these findings are summarized in Table 5.

#### **8. Future applications of D-galactose-induced brain aging model**

There are several types of aging models used in anti-aging studies such as X-ray induced aging, jet lag induced aging, naturally aging models and D-galactose-induced aging models (Yanar et al., 2011). Among these aging models, D-galactose-induced aging model has the least side effects and take shorter time than naturally aging. D-galactose can accelerate the aging process, and can be used in research which can be faster to induce aging and easily accessible to induce brain senescence (Aydin et al., 2012; Cebel et al., 2014; Chen et al., 2010; Cui et al., 2006; Lu et al., 2007; Qu et al., 2016). For example: 1) Aydin and colleagues demonstrated that D-galactose-induced aging rats had significant similarities with

the naturally aged rats in comparison of oxidative stress biomarkers in renal tissues (Aydin et al., 2012). 2) Cebe and colleagues also suggested that myocardial redox homeostasis in D-galactose-induced aging rats was similar to that in naturally aging rats (Cebe et al., 2014). 3) Chen and colleagues observed that age-related central auditory dysfunction and its corresponding pathological changes are also present in both naturally aging rats and the D-galactose mimetic aging model (Chen et al., 2010). 4) Moreover, D-galactose has been shown to effectively induce cognitive impairment and can mimic many characters of the natural brain aging process (Cui et al., 2006; Lu et al., 2007; Qu et al., 2016). Thus, these reports indicated that D-galactose can be used as a reliable animal model for mimetic aging.

Furthermore, various therapeutic interventions for brain aging which targeted on several pathways such as mitochondrial function, oxidative stress, apoptosis, inflammation and BDNF deficiency have shown promising results in animal studies (see Table 2 and 4). This information provides several mechanistic insights and can be beneficial for their clinical applications in elderly patients with brain aging disorders. For examples, as shown in D-galactose-induced aging animal models (Chen et al., 2016), hyperbaric therapy has been used as a method for resuscitation and therapy in elderly patients with acute cerebral and cardio-respiratory dysfunction (Rogatsky and Stambler, 2017). Moreover, anti-oxidant anthocyanin has been shown to improve beneficial effects for brain function and cognitive behavior in older people (Boespflug et al., 2017). In addition, the researchers started low-level laser therapy on the D-galactose induced aging animal model and now they observed that transcranial low-level laser therapy can restore ATP to delay cognitive decline in aging humans (de la Torre, 2017). All of these findings suggested that the therapeutic approaches used in D-galactose-induced aging model can be translated to clinical application for prevention and better treatments in the neurodegenerative disorders. This growing evidence suggests that D-galactose-aging model can be used for studies of aging and certain aging-related neurological

disorders, including Alzheimer's, Huntington's, and Parkinson's diseases. Moreover, several previous studies demonstrated that D-galactose could induce brain aging by causing oxidative stress and mitochondrial dysfunction (Banji et al., 2014; Kumar et al., 2009; Prakash and Kumar, 2013; Zhang et al., 2010). This will give a better understanding of the pathogenesis of cognitive decline and the nature of mitochondrial dysfunction during normal aging and in the early phases of neurodegenerative diseases for better therapeutic strategies.

## Conclusion

Aging is a world-wide problem we all are facing every day, which is why the therapeutic approaches to senescence are becoming more and more important. Of all the organs, the brain plays the most critical role in the process of senescence and is the most fragile of all the body organs in this instance. Brain aging encounters cognitive impairment which elevates the rate of dependency of older people worldwide. D-galactose-induced mimetic aging is associated with the involvement of mitochondrial dysfunction, inflammation and apoptosis and BDNF deficiency and has been shown to mediate cognitive impairment. The effect of D-galactose on mitochondrial dysfunction can be evaluated by determining the activity of respiratory enzymes, oxidative stress markers and ATP synthesis and also measure changes in mitochondria DNA mutations, mitochondrial membrane potential and mitochondrial structures. A variety of treatments which target mitochondria and reducing oxidative stress and inflammation could be the main therapeutic approaches to ameliorate the deterioration of brain aging exerted by D-galactose. The possible underlying mechanisms involved in D-galactose-induced brain aging and the possible therapeutic approaches utilized to date are summarized in Figure 1.

Therefore, the findings from this review, which is a comprehensive summary of previous studies investigating the D-galactose-induced aging model, could lead to a better

understanding of the underlying aging mechanisms. This increased knowledge could allow researchers to more-effectively manage the socio-economic burden of age-related disorders.

ACCEPTED MANUSCRIPT

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**Competing interests**

The authors declare that they have no competing interests.

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**Figure 1.** The possible outcomes of D-galactose-induced brain aging and the therapeutic approaches available for its treatment. SOD: superoxide dismutase;  $\text{H}_2\text{O}_2$ : hydrogen peroxide;  $\text{OH}^\cdot$ : hydroxide ion;  $\text{NH}_2$ : amino group;  $\text{Fe}^{+2}$ : ferrous oxide; AGE: advanced glycation end products; RAGE: receptors for advanced glycation end products; NADPH: Nicotinamide adenine dinucleotide phosphate; ROS: reactive oxygen species; ATP: Adenosine triphosphate; Cyt C: cytochrome c; JNK: phosphorylated c-Jun N-terminal kinase

**Table1: D-galactose-induced brain aging**

Study Model	Methods	Brain Area	Aging markers	Interpretation	Ref
<p>10-week-old Kunming mice</p> <p>Groups:</p> <p>(1) control (0.9 % saline, s.c)</p> <p>(2) D-gal (50 mg/kg/d, s.c)</p> <p>Duration: 8 weeks</p>	<p>- AGEs, RAGE by Immunosorbent Assay</p>	Prefrontal cortex	<ul style="list-style-type: none"> <li>• ↑ AGEs</li> <li>• ↑ RAGE</li> </ul>	D-gal-induced brain aging by increasing advanced glycation end products and its receptors.	(Lu et al., 2010b)
<p>3-month-old Kumming mice</p> <p>Groups:</p> <p>(1) control (0.9 % saline, s.c)</p> <p>(2) D-gal (180 mg/kg/d, inj)</p> <p>Duration: 8weeks</p>	<p>- AGE by ELISA</p> <p>- AR, SDH, RAGE by PCR</p>	Whole Brain	<ul style="list-style-type: none"> <li>• ↑ AGE</li> <li>• ↑ RAGE</li> <li>• ↑ AR</li> <li>• ↑ SDH</li> </ul>	D-gal-induced brain aging by increasing advanced glycation end products, its receptors, aldose reductase and sorbitol dehydrogenase.	(Yu et al., 2015)
<p>3-month-old Sprague-Dawley rats</p> <p>Groups:</p> <p>(1) control (0.9 % saline, s.c)</p>	<p>- SA-β-gal (Senescence associated β-galactosidase cytochemical staining)</p> <p>- telomere length by Southern blot</p> <p>- telomerase by TRAP-PCR</p>	Hippocampus	<p><b>- Senescence-associated genes:</b></p> <ul style="list-style-type: none"> <li>• ↑ p53</li> <li>• ↑ p19<sup>Arf</sup></li> </ul>	D-galactose-induced hippocampus senescence by regulating telomere length, senescence-associated genes, and function of lysosomes.	(Zhu et al., 2014)

Study Model	Methods	Brain Area	Aging markers	Interpretation	Ref
(2) D-gal (120 mg/kg/d, s.c)  Duration: 6 weeks	- p53, p19 <sup>Arf</sup> , p21 <sup>Cip1/Waf1</sup> by qRT-PCR		<ul style="list-style-type: none"> <li>• ↑p21<sup>Cip1/Waf1</sup></li> </ul> <p><b>- Function of the lysosomes:</b></p> <ul style="list-style-type: none"> <li>• ↑ SA-β-gal staining</li> <li>- ↓ telomere lengths</li> <li>- ↓ telomerase activity</li> </ul>		
3-month-old Sprague-Dawley rats Groups: (1) control (0.9 % saline, s.c) (2) D-gal (100 mg/kg/d, i.p.)  Duration: 7 weeks	- Aβ, BACE-1, RAGE by Western Blot - Aβ by Immunofluorescence Analysis	Whole Brain	<p><b>- Amyloid protein:</b></p> <ul style="list-style-type: none"> <li>• ↑ BACE-1</li> <li>• ↑ Aβ</li> <li>• ↑ RAGE</li> </ul>	D-galactose induced brain aging by increasing amyloid protein, beta-secretase 1 and advanced glycation end products receptor.	(Rehman et al., 2017)
3- month-old Kunming mice Groups: (1) Control	- p21, p53, Aβ <sub>1-42</sub> by Western Blot	Hippocampus	<ul style="list-style-type: none"> <li>• ↑ AGE</li> <li>• ↑ p16, p21, p53</li> <li>• ↑ Aβ<sub>1-42</sub></li> </ul>	D-galactose induced hippocampal senescence by increasing advanced glycation end products, its receptor and senescence-	(Chen et al., 2016)

Study Model	Methods	Brain Area	Aging markers	Interpretation	Ref
(2) D-gal (200 mg/kg/d, i.p)				associated genes.	
Duration: 8 weeks					
s.c: subcutaneous; AGEs: advanced glycation endproducts; RAGE: AGE receptors; inj: injection; ELISA: enzyme-linked immunosorbent assay; AR: aldose reductase; SDH: sorbitol dehydrogenase; PCR: polymerase chain reaction; SA- $\beta$ -gal: senescence-associated $\beta$ -galactosidase; TRAP-PCR: Telomeric repeat amplification protocol; p53, p19Arf, p21Cip1/Waf1: senescence-associated genes; qRT-PCR: qualitative real time PCR; A $\beta$ : amyloid beta protein; BACE-1: $\beta$ -site amyloid precursor protein cleaving enzyme 1; i.p: intraperitoneal					

**Table.2: D-galactose-induced mitochondrial dysfunction in aging models**

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Mitochondrial findings	Related findings		
2-month-old Shanghai C57BL/6J mice Groups: (1) control (0.9 % saline, s.c) (2) D-gal (100 mg/kg/d, s.c)  Duration: 6 weeks.	- Mitochondrial respiration by Clark oxygen electrode - Assay of mitochondrial enzyme activities and kinetics by spectrophotometer	Whole Brain	- <b>Oxidative phosphorylation:</b> • ↓ state 3 respiration • ↓ respiratory control ratio (RCR) • ↓ ADP/O ratio • ↔maximum velocity (Vmax) and substrate binding affinity (Km) of the complexes	( - )	D-galactose induced mitochondrial dysfunction by decreasing oxidative phosphorylation enzyme activity in the mitochondria of senescence mice brain.	(Long et al., 2007)
2–3 month-old Male Swiss albino mice Groups: (1) Naive (0.5 % sodium carboxymethyl cellulose, 0.5 ml/100 g/d, p.o)	- NADH dehydrogenase, succinate dehydrogenase activity, cytochrome oxidase, MTT ability, glutathione, catalase, superoxide dismutase, and glutathione-S-transferase activity, MDA and nitrite level, caspase3	Whole Brain (excluding cerebellum)	- <b>Oxidative phosphorylation:</b> • ↓ NADH dehydrogenase, succinate dehydrogenase activity, cytochrome oxidase	- <b>Antioxidant enzymes:</b> • ↓ glutathione, catalase, superoxide dismutase, and glutathione-S-transferase activity	D-galactose induced mitochondrial dysfunction and biochemical changes by causing oxidative stress, apoptosis and decreasing	(Kumar et al., 2009; Prakash and Kumar, 2013)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Mitochondrial findings	Related findings		
(2) D-gal (100 mg/kg/d, s.c)  Duration: 6 weeks	by spectrophotometer		- <b>Cell viability:</b> • ↓MTT ability	- <b>Oxidative stress:</b> • ↑ MDA, and nitrite level  - <b>Apoptosis:</b> • ↑ caspase 3	antioxidant enzymes, oxidative phosphorylation, and cell viability in senescence mice.	
3-month-old Kunming mice Groups: (1) control (0.9 % saline, s.c) (2) model (D-gal, 150 mg/kg/d, s.c)  Duration: 6 weeks	- Complex I, II, III, IV activity by spectrophotometer - Changes in MMP, ROS by fluorescent plate reader - TNOS, iNOS by commercially available kits.	Cerebral cortex and hippocampus	- <b>Oxidative phosphorylation:</b> • ↓ complex I, II, III, IV activity  - <b>Function:</b> • ↓ MMP	- <b>Oxidative stress:</b> • ↑ ROS, • ↑ TNOS and iNOS	D-galactose decreased the activity of mitochondria respiratory chain/oxidative phosphorylation system and cell viability by causing oxidative stress.	(Zhang et al., 2010)
1-month-old male Sprague–Dawley rats Groups: (1) control (0.9 % saline, s.c)	- Serum H <sub>2</sub> O <sub>2</sub> , T-SOD activity and MDA assay by colorimetric kits - Tissue H <sub>2</sub> O <sub>2</sub> assay by an Enhanced BCA Protein Assay Kit	Ventral cochlear nucleus	- <b>Structural changes:</b> • ↑ mtDNA common deletion (CD), • Swollen with a	- <b>Oxidative stress:</b> • ↑ H <sub>2</sub> O <sub>2</sub> • ↑ MDA, 8-OHdG • ↑ p22 <sup>phox</sup> , p47 <sup>phox</sup> ,	D-galactose induced the accumulation of mtDNA mutations, the decline of ATP and MMP and the	(Du et al., 2015)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Mitochondrial findings	Related findings		
(2) D-gal (L) 150 mg/kg/d, s.c, (3) D-gal (M) 300 mg/kg/d, s.c. (4) D-gal (H) 500 mg/kg/d, s.c,  Duration: 8 weeks	- NOX2, p22 <sup>phox</sup> , p47 <sup>phox</sup> , p67 <sup>phox</sup> by RT-PCR - NOX2 and 8-OHdG by immunohistochemical analysis - p22 <sup>phox</sup> , p47 <sup>phox</sup> , p67 <sup>phox</sup> , caspase 3, cyt c by Western blot - DNA isolation and determination of the mtDNA common deletion by Genomic DNA Purification Kit - ultrastructure of mitochondria by TEM - Detection of ATP levels by BCA assay kit - Measurement of MMP by fluorescent - apoptosis by TUNEL assay		reduced electron density in the matrix  <b>-Oxidative, phosphorylation:</b> • ↓ ATP  <b>-Function:</b> • ↓ MMP  -Note: All doses of D-galactose show the same effects.	p67 <sup>phox</sup> of NOX2  <b>-Antioxidant enz:</b> • ↓ T-SOD  <b>-Apoptosis:</b> • ↑ cyt. c and cleaved caspase-3  - ↑ TUNEL-positive cells	activation of caspase-3-dependent apoptosis in the central auditory system via increasing NOX2 expression.	
1-month-old Sprague–Dawley rats Groups: (1) control (0.9 % saline, s.c)	- DNA isolation and determination of mtDNA 4834 bp deletion by Genomic DNA Purification Kit - RNA preparation and	Auditory cortex	<b>-Structure:</b> • ↑ mtDNA common deletion (CD)  -Note: All doses of	<b>-DNA repair enzymes:</b> • ↓ OGG1, pol $\gamma$  - ↑ TUNEL-positive cells	D-galactose induced mtDNA damage resulted from decreased DNA repair enzymes and	(Chen et al., 2011)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Mitochondrial findings	Related findings		
(2) D-gal (150 mg/kg/d, s.c) (3) D-gal (300 mg/kg/d, s.c) (4) D-gal (500 mg/kg/d, s.c)  Duration: 8 weeks	quantitative RT-PCR - OGG1, pol $\gamma$ by Western blot - apoptosis by TUNEL staining		D-galactose show the same effects.		increased apoptosis leading to presbycusis in aging rats.	
5-week-old male Sprague-Dawley rats Groups: (1) the control group (0.9 % saline, s.c) (2) D-gal group (500 mg/kg/d, s.c)  Duration: 8 weeks	- quantity of the mtDNA CD by TaqMan real-time PCR - ultrastructure of the hippocampal mitochondria was observed under Transmission Electron Microscopy -NOX and UCP2 by Western Blot	Hippocampus	<b>-Structural changes:</b> <ul style="list-style-type: none"> <li>• <math>\uparrow</math> mtDNA common deletion (CD),</li> <li>• Significant swelling of all mitochondrial spaces, including cristae with a reduced electron density in the matrix.</li> </ul> <b>-Oxidative stress:</b> <ul style="list-style-type: none"> <li>• <math>\uparrow</math> UCP2</li> </ul>	<b>- Oxidative stress:</b> <ul style="list-style-type: none"> <li>• <math>\uparrow</math> p91<sup>phox</sup>, p22<sup>phox</sup>, p47<sup>phox</sup>, p67<sup>phox</sup> of NOX in hippocampus</li> </ul>	D-galactose induced brain mitochondria dysfunction, as indicated by common deletion of mitochondria DNA, impaired mitochondria structures in the hippocampus and increased oxidative stress, which mechanism may partly be related to NOX-dependent pathway.	(Du et al., 2012)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Mitochondrial findings	Related findings		
2-month-old Sprague Dawley rats Groups: (1) control (0.9 % saline, s.c) (2) D-gal (500 mg/kg/d, s.c) Duration: 8 weeks	- SOD, MDA by spectrophotometer - ultrastructure of mitochondria by Transmission electron microscopy - DNA extraction and cDNA generation by Genomic DNA Purification Kit - Quantification of mtDNA 4834 bp deletion by TaqMan real-time PCR assay - Gene expression analysis using real-time PCR - Sirt and SOD by Western blot and immunofluorescence - apoptosis by TUNEL staining	Auditory cortex	<b>-Structure changes:</b> <ul style="list-style-type: none"> <li>• ↑mtDNA CD, swollen mitochondria</li> </ul> <b>- Mitochondrial protein acetylation status:</b> <ul style="list-style-type: none"> <li>• ↓ mRNA expression of Sirt3</li> </ul>	<b>-Antioxidant enz:</b> <ul style="list-style-type: none"> <li>• ↓ SOD2</li> </ul> <b>- Oxidative stress:</b> <ul style="list-style-type: none"> <li>• ↑MDA</li> </ul> - enlarged endoplasmic reticulum and disrupted myelin - ↑ TUNEL-positive cells	D-galactose induced oxidative stress accumulated with aging, mitochondria dysfunction, abnormal ultrastructural changes and auditory cortex cell apoptosis.	(Zeng et al., 2014)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Mitochondrial findings	Related findings		
<p>4-month-old Wistar rats</p> <p>Groups:</p> <p>(1) control (methyl cellulose, 2 %, in distilled water, p.o)</p> <p>(2) D-gal (150 mg/kg/d, s.c)</p> <p>Duration:</p> <p>7weeks</p>	<p>- GSH, GPx, MDA, protein thiol (-SH) groups, AOPP (Advanced oxidation protein products), Acotinase, complex I, II, IV by spectrophotometer</p> <p>-caspase 3 by western blot</p> <p>- succinate dehydrogenase by spectroscopic techniques</p> <p>- complex III chromatography</p>	Whole Brain	<p>- <b>Oxidative phosphorylation:</b></p> <ul style="list-style-type: none"> <li>• ↓ NADH-co Q oxidoreductase (I), succinate-co Q oxidoreductase (II), co Q-cytochrome C oxidoreductase (III), cytochrome C oxidase (IV)</li> </ul> <p>- <b>Tricarboxylic acid cycle enzymes:</b></p> <ul style="list-style-type: none"> <li>• ↓ succinate dehydrogenase and aconitase</li> </ul>	<p>- <b>Antioxidant enz:</b></p> <ul style="list-style-type: none"> <li>• ↓ GSH, GPx</li> </ul> <p>- <b>Oxidative stress:</b></p> <ul style="list-style-type: none"> <li>• ↑ MDA, AOPP and protein carbonyls</li> </ul> <p>- <b>Apoptosis:</b></p> <ul style="list-style-type: none"> <li>• ↑ caspase-3</li> </ul> <p>- damage to neurons in CA1 region</p>	D-galactose induced mitochondrial oxidative phosphorylation, tricarboxylic acid cycle enzymes, biochemical changes and histological alterations.	(Banji et al., 2014)
<p>Middle-aged Sprague-Dawley rats</p> <p>Groups:</p> <p>(1) control (0.9 % saline, s.c)</p> <p>(2) Mimetic aging group (10</p>	<p>- Peripheral-type benzodiazepine binding sites by autoradiographic localization</p>	Cerebral cortex	<p>-<b>Antioxidant ability:</b></p> <ul style="list-style-type: none"> <li>• ↓ mitochondrial peripheral benzodiazepine receptors</li> </ul>	( - )	D-galactose decreased the antioxidant ability in mitochondria from cerebral cortex.	(Chen et al., 2008)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Mitochondrial findings	Related findings		
% D-gal, 1 ml/kg/d, s.c)						
Duration: 8 weeks						
s.c: subcutaneous; ADP/O: Phosphate/Oxygen Ratio; p.o: per oral; NADH: Nicotinamide adenine dinucleotide; MTT: tetrazolium dye; MDA: malondialdehyde; MMP: mitochondrial membrane potential; ROS: reactive oxygen species; TNOS: total nitric oxide synthase; iNOS: inducible nitric oxide synthase; H <sub>2</sub> O <sub>2</sub> : hydrogen peroxide; T-SOD: total superoxide dismutase; BCA: bicinchoninic acid; 8-OHdG = 8-hydroxy-2'-deoxyguanosine; ATP: Adenosine triphosphate; NOX: NADPH oxidase; p22 <sup>phox</sup> , p47 <sup>phox</sup> , p67 <sup>phox</sup> : subunit of NOX; cyt c: cytochrome c; DNA: Deoxyribonucleic acid; mtDNA: mitochondria DNA; TEM: transmission electron microscopy; TUNEL: terminal deoxynucleotidyl transferase dUTP nick end labeling; UCP2: uncoupling protein 2; cDNA: complementary DNA; bp: base pair; PCR: polymerase chain reaction; GSH: glutathione; GPx: glutathione peroxidase; co Q: coenzyme Q; OGG1 pol <sub>γ</sub> : 8-Oxoguanine glycosylase polymerase gamma						

**Table 3: Therapeutic approach on brain mitochondrial aging induced by D-galactose**

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Mitochondrial findings	Related findings		
<p>2-month-old Shanghai C57BL/6J mice</p> <p>Groups:</p> <p>(1) control (0.9 % saline, s.c)</p> <p>(2) normal treated with R-alpha-lipoic acid (LA),100 mg/kg/d, i.p</p> <p>(3) D-gal (100 mg/kg/d, s.c)</p> <p>(4) D-gal (100 mg/kg/d, s.c) + LA treatment (100 mg/kg/d, i.p)</p> <p>Duration: 6 weeks.</p>	<p>- Mitochondrial respiration by Clark oxygen electrode</p> <p>- Assay of mitochondrial enzyme activities and kinetics by spectrophotometer</p>	Whole Brain	<p><b>- Oxidative phosphorylation:</b></p> <ul style="list-style-type: none"> <li>• ↑ state 3 respiration</li> <li>• ↑respiratory control ratio(RCR)</li> <li>• ↓ADP/O ratio</li> <li>• ↔ maximum velocity (Vmax) and substrate binding affinity (Km)of complex III</li> </ul>	( - )	<p><b>R-alpha-lipoic acid</b> treatment ameliorated the D-gal-induced mitochondrial dysfunction by restoring the oxidative phosphorylation enzyme activity.</p>	(Long et al., 2007)
<p>2–3 month-old Male Swiss albino mice</p> <p>Groups:</p> <p>(1) Naive (0.5 % sodium</p>	<p>- NADH dehydrogenase, succinate dehydrogenase activity, MTT ability,</p>	Whole Brain (excluding cerebellum)	<p><b>-Oxidative phosphorylation:</b></p> <ul style="list-style-type: none"> <li>• ↑ NADH dehydrogenase,</li> </ul>	<p><b>-Oxidative stress:</b></p> <ul style="list-style-type: none"> <li>• ↓ MDA, and nitrite level</li> </ul>	<p><b>Carvidilol (CAR)</b> attenuate D-galactose-induced mitochondrial dysfunction, biochemical changes by increasing antioxidant</p>	(Kumar et al., 2009)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Mitochondrial findings	Related findings		
carboxymethyl cellulose, 0.5 ml/100 g/d, p.o) (2) D-gal (100 mg/kg/d, s.c) (3) CAR (5 mg/kg/d, p.o) (4) CAR (2.5 mg/kg/d, p.o) + D-gal (100 mg/kg/d, s.c) (5) CAR (5 mg/kg/d, p.o) + D-gal (100 mg/kg/d, s.c)  Duration: 6 weeks	glutathione, catalase, superoxide dismutase, and glutathione-S-transferase activity, MDA, and nitrite level by spectrophotometer		succinate dehydrogenase activity  <b>- Cell viability:</b> • ↑MTT ability  - Note: High dose CAR (5mg/kg/d) was more effective than low dose CAR (2.5mg/kg/d) in all parameters.	<b>-Antioxidant enz:</b> • ↑ glutathione, catalase, superoxide dismutase, and glutathione-S-transferase activity	enzymes, oxidative phosphorylation, and cell viability in senescence mice.	
3-month-old Male Laca mice Groups: (1) Naïve (0.5 % sodium carboxymethyl cellulose, 1 ml/100 g/d, p.o) (2) D-gal (100 mg/kg/d, s.c)	- NADH dehydrogenase, succinate dehydrogenase activity, cytochrome oxidase, MTT ability, glutathione, catalase, superoxide dismutase, and glutathione-S-transferase activity,	Whole Brain (excluding cerebellum)	<b>- Oxidative phosphorylation:</b> • ↑NADH dehydrogenase, succinate dehydrogenase, cytochrome oxidase	<b>- Antioxidant enz:</b> • ↑ glutathione, catalase, superoxide dismutase, and glutathione-S-	<b>Pioglitazone (PIO)</b> attenuates D-galactose-induced mitochondrial dysfunction, oxidative stress and apoptosis by increasing antioxidant enzymes, oxidative phosphorylation, and cell viability in senescence mice through activation	(Prakash and Kumar, 2013)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Mitochondrial findings	Related findings		
(3) PIO (10 mg/kg/d, p.o) (4) PIO (30 mg/kg/d, p.o) (5) PIO (10 mg/kg/d, p.o) + D-gal (100 mg/kg/d, s.c) (6) PIO (30 mg/kg/d, p.o) + D-gal (100 mg/kg/d, s.c) (7) bisphenol A diglycidyl ether (BADGE) (PPAR <sub>γ</sub> antagonist) (15 mg/kg/d, p.o) + PIO (30 mg/kg/d, p.o) + D-gal (100 mg/kg/d, s.c)  Duration: 6 weeks	MDA, and nitrite level, caspase 3 by spectrophotometer		<b>- Cell viability:</b> <ul style="list-style-type: none"> <li>↑ MTT ability</li> </ul> <b>- BADGE treatment</b> blocked the protective effect of pioglitazone. - Note: High dose - PIO (30mg/kg/d) was more effective than low dose PIO (10mg/kg/d) in all parameters except in caspase 3 activity.	transferase activity  <b>-Oxidative stress:</b> <ul style="list-style-type: none"> <li>↓ MDA, nitrite level</li> </ul> <b>-Apoptosis:</b> <ul style="list-style-type: none"> <li>↓ caspase-3</li> </ul>	of PPAR <sub>γ</sub> receptors.	
3-month-old	- Complex IV activity	Cerebral	- Oxidative	-Oxidative	Catalpol could ameliorate	(Zhan

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Mitochondrial findings	Related findings		
<p>Kunming mice</p> <p>Groups:</p> <p>(1) control (0.9 % saline, s.c)</p> <p>(2) model (D-gal, 150 mg/kg/d, s.c)</p> <p>(3) catalpol (2.5 mg/kg/d, s.c) + D gal (150 mg/kg/d, s.c)</p> <p>(4) catalpol (5 mg/kg/d, s.c) + D gal (150 mg/kg/d, s.c)</p> <p>(5) catalpol (10 mg/kg/d, s.c) + D gal (150 mg/kg/d, s.c)</p> <p>Duration: 6 weeks</p>	<p>by spectrophotometer</p> <p>- Changes in MMP, ROS by fluorescent plate reader</p> <p>- TNOS, iNOS by commercially available kits.</p>	<p>cortex and hippocampus</p>	<p><b>phosphorylation:</b></p> <ul style="list-style-type: none"> <li>• ↑ complex I, II, III, IV activity</li> </ul> <p>- <b>Cell viability:</b></p> <ul style="list-style-type: none"> <li>• ↑ MMP</li> </ul> <p>-Note: All doses of Catapol show the same effects.</p>	<p><b>stress:</b></p> <ul style="list-style-type: none"> <li>• ↓ ROS</li> <li>• ↓ TNOS and iNOS</li> </ul>	<p>D-galactose induced mitochondrial dysfunction and biochemical changes by decreasing oxidative stress.</p>	<p>g et al., 2010)</p>
<p>Adult Male BALB/c mice</p> <p>Groups:</p> <p>(1) control (0.9 % saline, s.c)</p> <p>(2) D-gal +sham</p>	<p>- Active mitochondria levels by MitoTracker Green staining</p> <p>- Mitochondrial membrane potential determination by</p>	<p>Whole brain</p>	<p>- ↑ <b>Active mitochondrial levels</b></p> <p>- <b>Oxidative phosphorylation:</b></p> <ul style="list-style-type: none"> <li>• ↑ cytochrome C</li> </ul>	<p>-<b>Oxidative stress:</b></p> <ul style="list-style-type: none"> <li>• ↓ ROS</li> </ul> <p>- <b>Apoptosis:</b></p> <ul style="list-style-type: none"> <li>• ↓ Bax/Bcl-2</li> </ul>	<p><b>Transcranial low level laser therapy (LLLT)</b></p> <p>increased active mitochondria levels, membrane potential, ATP production and abrogated</p>	<p>(Saleh pour et al., 2017)</p>

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Mitochondrial findings	Related findings		
(500 mg/kg/d, s.c) (3) D-gal + red 4 (D-gal +4 J/cm <sup>2</sup> of red laser) (4) D-gal+ NIR 4 (D-gal+ 4 J/cm <sup>2</sup> of NIR laser) (5) D-gal+ red 8 (D-gal+ 8 J/cm <sup>2</sup> of red laser) (6) D-gal+ NIR 8 (D-gal+ 8 J/cm <sup>2</sup> of NIR laser)  Duration: 6 weeks	Mitochondria Staining Kit - Mitochondrial cytochrome c oxidase activity by commercial kits - Bax/Bcl-2, Caspase 3 by western blot - ATP levels by Colorimetric assay kit - ROS by Fluorescent dye dichlorohydrofluorescein diacetate - Barnes Maze test - What-Where-Which task		oxidase (IV) • ↑ ATP  - <b>Function:</b> • ↑ MMP	ratio • ↓ Caspase-3  - Note: Higher dose of LLLT exerted a better response than low dose (4 J/cm <sup>2</sup> ).	oxidative stress, apoptosis leading to restore mitochondrial dysfunction in D-galactose-induced aging mice.	
4-month-old Wistar rats Groups: (1) Control (methyl cellulose, 2 %, in distilled water, p.o) (2) D-gal (150 mg/kg/d, s.c) (3) Curcumin (50 mg/kg/d, p.o) + D-	- GSH, GPx, MDA, protein thiol (-SH) groups, AOPP (Advanced oxidation protein products), Acotinase, complex I, II, IV by spectrophotometer -caspase 3 by western blot	Whole Brain	- <b>Oxidative phosphorylation:</b> • ↑NADH-co Q oxidoreductase (I), succinate-co Qoxidoreductase (II), co Q-cytochrome C oxidoreductase (III), cytochrome C	- <b>Antioxidant enz:</b> • ↑ GSH, GPx  - <b>Oxidative stress:</b> • ↓ MDA, AOPP and protein carbonyls	<b>Curcumin and hesperidin (HES)</b> attenuated D-galactose induced mitochondrial dysfunction and apoptosis by increasing oxidative phosphorylation enzymes and tricarboxylic acid cycle enzymes and improve the functional capacity of neurons.	(Banji et al., 2014)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Mitochondrial findings	Related findings		
gal (150 mg/kg/d, s.c) (4) HES (10 mg/kg/d) + D-gal (150 mg/kg/d, s.c) (5) Curcumin (50 mg/kg/d) + HES (10 mg/kg/d) + D-gal (150 mg/kg/d, s.c) (6) Curcumin (100 mg/kg/d) + HES (25 mg/kg) + D-gal (150 mg/kg/d, s.c)  Duration: 7 weeks	- succinate dehydrogenase by spectroscopic techniques- - complex III by chromatography-techniques		oxidase (IV)  - <b>Tricarboxylic acid cycle enzymes:</b> • ↑ succinate dehydrogenase & aconitase  - Note: Higher dose of the combination of curcumin with HES exerted a better response than low dose combination and individual therapy.	- <b>Apoptosis:</b> • ↓ caspase-3		
Middle-aged Sprague-Dawley rats Groups: (1) control (0.9 % saline, s.c) (2) Mimetic aging group (10 % D-gal, 1 ml/kg/d, s.c) (3) 2 % DHEA-treated normal	- Peripheral-benzodiazepine Binding by autoradiographic localization	Cerebral cortex	- <b>Antioxidant ability:</b> • ↑ mitochondrial peripheral benzodiazepine receptors	( - )	<b>Dehydroepiandrosterone (DHEA)</b> attenuated D-galactose induced mitochondrial dysfunction by strengthening the antioxidant ability.	(Chen et al., 2008)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Mitochondrial findings	Related findings		
group (1 ml/kg/d, i.p) (4) Vehicle control group (2 % DMSO, i.p) + 10 % D-gal (1 ml/kg/d, s.c) - (5) 2 % DHEA-treated senescent group (1 ml/kg/d, i.p) + 10 % D-gal, (1 ml/kg/d, s.c) Duration: 8 weeks						
D-gal: D-galactose; s.c: subcutaneous; i.p: intraperitoneal; ADP/O: Phosphate/Oxygen Ratio; p.o: per oral; NADH: Nicotinamide adenine dinucleotide; MTT: tetrazolium dye; MDA: malondialdehyde; MMP: mitochondrial membrane potential; ROS: reactive oxygen species; TNOS: total nitric oxide synthase; iNOS: inducible nitric oxide synthase; H2O2: hydrogen peroxide; T-SOD: total superoxide dismutase; BCA: bicinchoninic acid; 8-OHdG: 8-hydroxy-2'-deoxyguanosine; ATP: Adenosine triphosphate; NOX: NADPH oxidase; p22phox, p47phox, p67phox: subunit of NOX; cyt c: cytochrome c; DNA: Deoxyribonucleic acid; mtDNA: mitochondria DNA; TEM: transmission electron microscopy; TUNEL: terminal deoxynucleotidyl transferase dUTP nick end labeling; UCP2: uncoupling protein 2; cDNA: complementary DNA; bp: base pair; PCR: polymerase chain reaction; GSH: glutathione; GPx: glutathione peroxidase; co Q: coenzyme Q; PPAR γ: peroxisome proliferator-activated receptor gamma; DMSO: dimethyl sulfoxide						



Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
10-week-old Kunming mice Groups: (1) control (0.9% saline, s.c) (2) D-gal (50 mg/kg/d, s.c)  Duration: 8 weeks	- Morris Water Maze Test - Step-through passive avoidance task - NF- $\kappa$ B, COX-2, iNOS, TNF $\alpha$ , IL-1 $\beta$ , IL-6 by Western Blot - AGEs, RAGE by Immunosorbent Assay - GFAP, CD11b by Immunofluorescence Staining - ROS, protein carbonyl by chromatography	prefrontal cortex	- <b>Morris water maze:</b> • $\uparrow$ Escape latency • $\downarrow$ Number of crossing • $\downarrow$ Time spent in the target quadrant  - <b>Step-through passive avoidance task:</b> • $\downarrow$ Latency time	- <b>Oxidative stress:</b> • $\uparrow$ Protein Carbonyl • $\uparrow$ ROS  - <b>Inflammatory markers:</b> • $\uparrow$ COX-2, iNOS, TNF $\alpha$ , IL-1 $\beta$ , IL-6, NF- $\kappa$ B  - <b>Aging marker:</b> • $\uparrow$ AGEs, RAGE  - <b>Activated astrocytes &amp; microglia cells:</b> • $\uparrow$ GFAP, CD11b	induced spatial learning and memory impairment via increasing oxidative stress, inflammation, aging process, and activated astrocytes and microglial cells.	al., 2010b)
2–3 month-old Male Swiss albino mice Groups: (1) Naïve (0.5 % sodium carboxymethyl	- Spatial navigation task (Morris water maze task) - Maze acquisition phase (training) - Maze retention phase (testing for retention of	Whole Brain (excluding cerebellum)	- <b>Morris water maze:</b> • $\leftrightarrow$ initial acquisition latency • $\uparrow$ first retention latency	- <b>Antioxidant enzymes:</b> • $\downarrow$ glutathione, catalase, superoxide dismutase, and glutathione-S-	D-galactose induced spatial learning and memory impairment, oxidative stress and apoptosis by	(Kumar et al., 2009; Prakash and Kumar, 2013)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
cellulose, 0.5 ml/100 g/d, p.o) (2) D-gal (100 mg/kg/d, s.c)  Duration: 6 weeks	the learned task) - Elevated plus maze paradigm - Assessment of gross behavioral activity - glutathione, catalase, superoxide dismutase, and glutathione-S-transferase activity, MDA, and nitrite level, caspase3, AchE activity by spectrophotometer		<ul style="list-style-type: none"> <li>• ↑ second retention latency</li> </ul> <p><b>- Elevated plus maze paradigm</b></p> <ul style="list-style-type: none"> <li>• ↔ initial transfer latency</li> <li>• ↑ first retention transfer latency</li> <li>• ↑ second retention transfer latency</li> <li>- ↔ locomotor activity</li> </ul>	<p>transferase activity</p> <p><b>-Oxidative stress:</b></p> <ul style="list-style-type: none"> <li>• ↑ MDA, and nitrite level</li> </ul> <p><b>- Neuromodulation:</b></p> <ul style="list-style-type: none"> <li>• ↑ AchE</li> </ul> <p><b>- Apoptosis:</b></p> <ul style="list-style-type: none"> <li>• ↑ caspase 3</li> </ul>	decreasing antioxidant enzymes, oxidative phosphorylation, and cell viability in senescence mice.	
3-month-old Kummig mice Groups: (1) control (0.9 % NaCl, s.c) (2) D-gal (180 mg/kg/d, s.c)  Duration: 8weeks	<p>- Water-maze test</p> <p>- Step-down test</p> <p>- ROS by spectroflurometer</p> <p>- AGE by ELISA</p> <p>- MDA, SOD, GPx, CAT, T-AOC by spectrophotometer</p> <p>- T-ChE by commercial kit</p> <p>- AR, SDH, RAGE, TNF-α, IL-6 by PCR</p>	Whole Brain	<p><b>- Water maze test:</b></p> <ul style="list-style-type: none"> <li>• ↑ Escape latency</li> <li>• ↓ Number of touching the blind</li> </ul> <p><b>- Step-down test:</b></p> <ul style="list-style-type: none"> <li>• ↑ number of errors</li> <li>• ↓ step down</li> </ul>	<p><b>-Oxidative stress:</b></p> <ul style="list-style-type: none"> <li>• ↑ MDA</li> </ul> <p><b>- Inflammatory markers:</b></p> <ul style="list-style-type: none"> <li>• ↑ NF-KB, TNF-α &amp; IL-6</li> </ul> <p><b>- Antioxidant enz:</b></p> <ul style="list-style-type: none"> <li>• ↓ SOD, GPx, CAT, T-AOC</li> </ul>	D-gal-induced spatial learning and memory impairment by causing oxidative stress, inflammation and reducing the activities of antioxidant enzymes and increasing AGE	(Yu et al., 2015)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
	- NF-KB by Western Blot		latency	<p><b>- Aging marker:</b></p> <ul style="list-style-type: none"> <li>• ↑ AGE, AR, SDH, RAGE</li> </ul> <p><b>- Neuromodulation:</b></p> <ul style="list-style-type: none"> <li>• ↑ T-chE</li> </ul> <p><b>- Histopathological changes</b></p> <ul style="list-style-type: none"> <li>• intracellular oedema, degeneration and necrosis of neurons, and hyperplasia in hippocampus</li> </ul>	formation, TChE activity.	
3-month-old Male Sprague-Dawley rats Groups: 1. control (0.9 % saline, s.c) 2. D-gal (120 mg/kg/d, s.c)	- Morris water maze test - Step-down type passive avoidance test - IL-1 $\beta$ , TNF- $\alpha$ , IL-6 by ELISA - Txnip, p-NF- $\kappa$ Bp65, p-I $\kappa$ B $\alpha$ , p-IKK $\alpha$ , p-IKK $\beta$ , Bax/Bcl-2 ratio,	Hippocampus	<p><b>- Morris water maze:</b></p> <ul style="list-style-type: none"> <li>• ↑ Escape latency</li> <li>• ↓ Number of crossing</li> <li>• ↓ Time spent in the target quadrant</li> </ul>	<p><b>- Inflammatory markers:</b></p> <ul style="list-style-type: none"> <li>• ↑ TNF-<math>\alpha</math>, IL-6, IL-1<math>\beta</math></li> <li>• ↓ Txnip</li> <li>• ↑ p-NF-<math>\kappa</math>Bp65, p-I<math>\kappa</math>B<math>\alpha</math>, p-IKK<math>\alpha</math>, p-IKK<math>\beta</math></li> </ul>	D-gal-induced spatial learning and memory impairment by causing inflammation and apoptosis.	(Gao et al., 2015)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
Duration: 6 weeks	caspase-9 by Western blot		<b>- Step-down test:</b> <ul style="list-style-type: none"> <li>• ↑ number of errors</li> <li>• ↓ step down latency</li> </ul>	<b>- Apoptosis:</b> <ul style="list-style-type: none"> <li>• ↑ Bax/Bcl-2 ratio</li> <li>• ↑ Caspase-9</li> </ul>		
3- month-old Kunming mice Groups: (1) Control (2) D-gal (200 mg/kg/d, i.p)  Duration: 8 weeks	- Morris water Maze test - p21, p53, Aβ <sub>1-42</sub> by Western Blot - AGEs, TNF-α, IL-6, SOD, GSH-Px, MDA, CAT, T-AOC, NO and NOS by commercial kits - TNF-α, IL-6, AGEs by ELISA - p16, p21, p53 by RT-PCR - immunoreactive cells for BDNF by Immunohistochemical analysis	Hippocampus	<b>- Morris water maze:</b> <ul style="list-style-type: none"> <li>• ↑ Escape latency</li> <li>• ↓ Time spent in the target quadrant</li> <li>• ↓ Number of crossing</li> </ul>	<b>-Oxidative stress:</b> <ul style="list-style-type: none"> <li>• ↑ MDA</li> <li>• ↑ NO levels</li> <li>• ↑ NOS</li> </ul> <b>- Antioxidant enz:</b> <ul style="list-style-type: none"> <li>• ↓ SOD,</li> <li>• ↓ GSH-Px</li> <li>• ↓ CAT</li> <li>• ↓ T-AOC</li> </ul> <b>- Inflammatory markers:</b> <ul style="list-style-type: none"> <li>• ↑ TNF-α, IL-6</li> </ul> <b>- Aging marker:</b> <ul style="list-style-type: none"> <li>• ↑ AGE</li> <li>• ↑ p16, p21, p53</li> <li>• ↑ Aβ<sub>1-42</sub></li> </ul>	D-galactose impaired the spatial learning and memory and hippocampal senescence by reducing the hippocampal BDNF expression, anti-oxidation, anti-inflammation and modulation of aging-related gene expression in hippocampus of mice.	(Chen et al., 2016)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
3-month-old Sprague-Dawley rats Groups: (1) control (0.9 % saline, s.c) (2) D-gal (120 mg/kg/d, s.c)  Duration: 6 weeks	- Morris Water Maze Test - GSH-px activity and GSH content, SOD activity and MDA by spectrophotometer - Detection of proinflammatory cytokines by ELISA - SA- $\beta$ -gal (Senescence associated $\beta$ - galactosidase cytochemical staining) - SOX2 by Western blot - telomere length by Southern blot - telomerase by TRAP-PCR - p53, p19 <sup>Arf</sup> , p21 <sup>Cip1/Waf1</sup> by qRT-PCR	Hippocampus	- <b>Morris water maze:</b> • $\uparrow$ Escape latency • $\downarrow$ Number of crossing • $\downarrow$ Time spent in the target quadrant	- $\downarrow$ BDNF positive cells - $\downarrow$ surviving neurones  - <b>Oxidative stress:</b> • $\uparrow$ MDA - <b>Antioxidant enz:</b> • $\downarrow$ GSH, SOD  - <b>Inflammatory markers:</b> • $\uparrow$ TNF- $\alpha$ , IL-6, IL-1 $\beta$  - <b>Hippocampal neurogenesis:</b> • $\downarrow$ SOX2 • $\downarrow$ BrdU cells  - <b>Senescence-associated genes:</b> • $\uparrow$ p53, p19 <sup>Arf</sup> , p21 <sup>Cip1/Waf1</sup>  - <b>Function of the lysosomes:</b> • $\uparrow$ SA- $\beta$ -gal	D-galactose induced spatial learning and memory impairment through effecting oxidative stress, antioxidant enzymes, inflammatory markers, hippocampus neurogenesis, senescence-associated genes, function of lysosomes, neuronal marker, activation of astrocytes, telomere length and telomerase activity.	(Zhu et al., 2014)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
				staining		
				<b>- Neuronal marker:</b> <ul style="list-style-type: none"> <li>• ↓ <math>\beta</math>-tubulin III</li> </ul>		
				<b>- Activated astrocytes:</b> <ul style="list-style-type: none"> <li>• ↑ Gal-c and GFAP</li> <li>• ↑ Nestin and Aeg1</li> </ul>		
				- ↓ telomere lengths, telomerase activity		
3-month-old Sprague-Dawley rats Groups: (1) control (0.9 % saline, s.c) (2) D-gal (100 mg/kg/d, i.p.) Duration: 7	- Morris Water Maze Test - Y-Maze Test - A $\beta$ , BACE-1, RAGE, TNF $\alpha$ , NF- $\kappa$ b, iNOS, Bax, Bcl-2, PARP-1, synaptophysin, syntaxin, SNAP-23, p-CREB by Western Blot	Whole Brain	<b>- Morris water maze:</b> <ul style="list-style-type: none"> <li>• ↑ Escape latency</li> <li>• ↓ Number of crossing</li> <li>• ↓ Time spent in the target quadrant</li> <li>• ↓ Swimming speed</li> </ul>	<b>- Oxidative stress:</b> <ul style="list-style-type: none"> <li>• ↑ MDA</li> <li>• ↑ 8-OxoG</li> <li>• ↑ ROS</li> </ul> <b>- Inflammatory markers:</b> <ul style="list-style-type: none"> <li>• ↑ iNOS, TNF<math>\alpha</math>, NF-<math>\kappa</math>B</li> </ul>	D-galactose induced spatial learning and memory impairment through oxidative stress, neuroinflammation, apoptosis, reducing synaptic proteins, increasing amyloid	(Rehman et al., 2017)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
weeks	- ROS by spectrofluorometer - MDA by fluorometric assay kit - 8-OxoG, p-JNK, GFAP, Iba-1, A $\beta$ by Immunofluorescence Analysis		- <b>Y-Maze Test</b> • ↓ The percentage of spontaneous alteration	- <b>Apoptosis:</b> • ↑ Bax/Bcl-2 • ↑ p-JNK • ↑ PARP-1  - <b>Synaptic proteins:</b> • ↓ synaptophysin, syntaxin, SNAP-23, p-CREB  - <b>Amyloid protein:</b> • ↑ BACE-1, A $\beta$  - <b>Aging marker:</b> • ↑ RAGE  - <b>Activated astrocytes &amp; microglia cells:</b> • ↑ GFAP, Iba-1  - <b>Antioxidant enz:</b> • ↓ GSH, GPx	protein and activation of astrocytes, microglial cells.	
4-month-old Wistar rats	- Morris water maze task	Whole Brain	- <b>Morris water maze:</b>		D-galactose induced significant	(Banji et al.,

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
<p>Groups:</p> <p>(1) control (methyl cellulose, 2 %, in distilled water, p.o)</p> <p>(2) D-gal (150 mg/kg/d, s.c)</p> <p>Duration: 9 weeks</p>	<p>- GSH, GPx, MDA, protein thiol (-SH) groups, AOPP (Advanced oxidation protein products) by spectrophotometer</p> <p>-caspase 3 by Electrophoresis and western blot</p>		<ul style="list-style-type: none"> <li>• ↓Number of crossing</li> <li>• ↓ Time spent in the target quadrant</li> </ul>	<p>- <b>Oxidative stress:</b></p> <ul style="list-style-type: none"> <li>• ↑ MDA, AOPP and protein carbonyls</li> </ul> <p>- <b>Apoptosis:</b></p> <ul style="list-style-type: none"> <li>• ↑ caspase-3</li> </ul> <p>- damage to neurons in CA1 region</p>	<p>spatial learning and memory deficits via increasing oxidative stress, apoptosis and histological alterations.</p>	<p>2014)</p>
<p>Adult Sprague Dawley rats</p> <p>Groups</p> <p>(1) control (0.9 % saline, s.c)</p> <p>(2) D-gal 120 mg/kg/d, i.p)</p> <p>Duration:60 D</p>	<p>- Y-maze task</p> <p>- PJNK, COX-2, NOS 2, IL-1<math>\beta</math>, TNF-<math>\alpha</math>, Cyt.C, PARP-1, Bax/Bcl-2, Caspase3, Caspase9, synaptophysin and PSD95 by Western Blot</p> <p>- PJNK, Caspase3, 8-oxoguanine by immunofluorescence analysis</p> <p>- Degenerating neurons by Fluoro-Jade B staining</p> <p>- Survival neurons by Cresyl violet staining</p>	<p>Hippocampus, Cortex</p>	<p>- <b>Y-Maze Test</b></p> <ul style="list-style-type: none"> <li>• ↓ The percentage of spontaneous alteration</li> </ul>	<p>- <b>Synaptic proteins:</b></p> <ul style="list-style-type: none"> <li>• ↓ synaptophysin, PSD95</li> </ul> <p>- <b>Oxidative stress:</b></p> <ul style="list-style-type: none"> <li>• ↑ 8-oxoguanine</li> </ul> <p>- <b>Inflammatory markers:</b></p> <ul style="list-style-type: none"> <li>• ↑ COX-2, NOS-2, TNF<math>\alpha</math> &amp; IL-1<math>\beta</math></li> </ul> <p>- <b>Apoptosis:</b></p>	<p>D-galactose induced spatial learning and memory impairment via increasing synaptic dysfunction, oxidative stress, apoptosis, neuroinflammation and neurodegeneration.</p>	<p>(Ullah et al., 2015)</p>

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
				<ul style="list-style-type: none"> <li>• ↓ anti-apoptotic Bcl2</li> <li>• ↑ ap Bax</li> <li>• ↑ Caspase-9, Caspase-3</li> <li>• ↑ PARP-1</li> <li>• ↑ p-JNK</li> </ul> <p><b>- Degenerating neurons:</b></p> <ul style="list-style-type: none"> <li>• ↑ FJB + neuronal cells</li> </ul> <p><b>- Survival neurons:</b></p> <ul style="list-style-type: none"> <li>• ↓ Cresyl violet neurones</li> </ul>		
Adult Male Wistar rats Groups: (1) control group (0.9 % saline, s.c) (2) D-gal (100 mg/kg/d, s.c) Duration: 8	- Morris water maze test - Open field test - MDA, NO, GSH, ACh, GPx, SOD, CAT, AChE by commercial kit - NF-κB, caspase-3, GFAP by Immunohistochemical	Whole Brain	- <b>Morris water maze:</b> <ul style="list-style-type: none"> <li>• ↑ Escape latency</li> <li>• ↓ Number of crossing</li> <li>• ↓ Time spent in the target quadrant</li> <li>• ↔Swimming</li> </ul>	- <b>Oxidative stress:</b> <ul style="list-style-type: none"> <li>• ↑ MDA</li> <li>• ↑ NO levels</li> </ul> <p><b>- Antioxidant enz:</b></p> <ul style="list-style-type: none"> <li>• ↓ GSH, SOD, CAT and GPx</li> </ul> <p>-</p>	D-galactose induced significant spatial learning and memory and locomotor and behavioral activity impairment, neurochemical deficits through oxidative damage,	(Qu et al., 2016)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
weeks.	analysis - Histopathological analysis		speed  - <b>Open field test:</b> • ↓ Number of grid crossing • ↓ Number of rearing and learning	<b>Neuromodulation:</b> • ↑ AChE • ↓ ACh  - <b>Apoptosis:</b> • ↑ Caspase-3  - <b>Inflammatory markers:</b> • ↑ NF-κB  - extensively damaged neurons in the hippocampus CA1 region	dysfunction of the cholinergic system, inflammatory markers and activation of astrocytes.	
Middle-aged Sprague-Dawley rats Groups: (1) control (0.9 % saline, s.c) (2) Mimetic aging group (D-gal, 10 % for stock solution, 1 ml/kg/d, s.c)	- Morris water maze test - Peripheral-type benzodiazepine binding sites by autoradiographic localization	Cerebral cortex	- <b>Morris water maze:</b> • ↑ Escape latency • ↓ Time spent in the target quadrant • ↓ Swimming distance	- <b>Antioxidant ability:</b> • ↓ mitochondrial peripheral benzodiazepine receptors	D-galactose impaired the spatial learning and memory impairment by decreasing the antioxidant ability in mitochondria from cerebral cortex.	(Chen et al., 2008)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		

Duration: 8 weeks

s.c: subcutaneous; p.o: per oral; NADH: Nicotinamide adenine dinucleotide; MDA: malondialdehyde; NO: nitric oxide; SOD: superoxide dismutase; AchE: acetyl choline esterase; IL: interleukin; NF- $\kappa$ B: nuclear factor kappa-light-chain-enhancer of activated B cells; ELISA: enzyme-linked immunosorbent assay; COX: Cyclooxygenase; TNF: tumor necrosis factor alpha; GFAP: Glial fibrillary acidic protein; ROS: reactive oxygen species; TNOS: total nitric oxide synthase; iNOS: inducible nitric oxide synthase; 8-OHdG: 8-hydroxy-2'-deoxyguanosine; Ach E: acetylcholinesterase; NOX: NADPH oxidase; p22<sup>phox</sup>, p47<sup>phox</sup>, p67<sup>phox</sup>: subunit of NOX; cyt c: cytochrome c; bp: base pair; PCR: polymerase chain reaction; TRAP-PCR: Telomeric repeat amplification protocol; GSH: glutathione; GPx: glutathione peroxidase; CAT: catalase; T-AOC: total antioxidant capacity; AGEs: advanced glycation endproducts; RAGE: AGE receptors; PCR: polymerase chain reaction; SA- $\beta$ -gal: senescence-associated  $\beta$ -galactosidase; Txnip: thioredoxin-interacting protein; p-IKK $\alpha$ , p-IKK $\beta$  phosphorylated I $\kappa$ B kinase  $\alpha$ ,  $\beta$ ; Bcl: B-cell lymphoma; Bax: bcl-2-like protein 4; SOX: SRY (sex determining region Y)-box 2; p53, p19<sup>Arf</sup>, p21<sup>Cip1/Waf1</sup>: senescence-associated genes; BrdU: Bromodeoxyuridine; Gal-c: galactosylceramidase; Aeg1: astrocyte elevated gene-1; A $\beta$ : amyloid beta protein; BACE-1:  $\beta$ -site amyloid precursor protein cleaving enzyme 1; PARP: poly (ADP-ribose) polymerase; SNAP-23: synaptosomal-associated protein 23; p-CREB: cAMP response elements binding; p-JNK: phosphorylated c-Jun N-terminal kinases; Iba-1: ionized calcium-binding adapter molecule 1; PSD95: postsynaptic density-95

**Table 5: Therapeutic approach on cognitive function in D-galactose-induced aging models**

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
8-10-week-old Kunming mice Groups: (1) control (0.9 % saline, s.c) (2) D-gal (150 mg/kg/d, s.c) (3) D-gal (150 mg/kg/d, s.c) + FA (50 mg/kg/d, i.g) (4) D-gal (150 mg/kg/d, s.c) + FA (100 mg/kg/d, i.g)  Duration: 8 weeks	- Morris water maze test - Open field test - MDA, NO, GSH, SOD, AChE by commercial kit - IL-1 $\beta$ , NF- $\kappa$ B by ELISA - H&E and Nissl staining - Immunohistochemistry	Whole Brain	<b>- Morris water maze:</b> <ul style="list-style-type: none"> <li>• ↓ Escape latency</li> <li>• ↑ Number of crossing</li> <li>• ↑ Time spent in the target quadrant</li> <li>• ↔Swimming speed</li> </ul> <b>- Open field test:</b> <ul style="list-style-type: none"> <li>• ↑ Number of grid crossing</li> <li>• ↑ Number of rearing and learning</li> <li>• ↑ Number of grooming</li> </ul> - Note: High dose FA (100mg/kg/d)	<b>-Oxidative stress:</b> <ul style="list-style-type: none"> <li>• ↓ MDA</li> <li>• ↓ NO levels</li> </ul> <b>- Antioxidant enz:</b> <ul style="list-style-type: none"> <li>• ↑GSH, SOD</li> </ul> <b>- Neuromodulation:</b> <ul style="list-style-type: none"> <li>• ↓ AChE</li> </ul> <b>- Apoptosis:</b> <ul style="list-style-type: none"> <li>• ↓ Caspase-3</li> </ul> <b>- Inflammatory markers:</b> <ul style="list-style-type: none"> <li>• ↓ NF-<math>\kappa</math>B, IL-1<math>\beta</math></li> </ul> - prevent neuronal cell damage to the hippocampus	<b>Ferulic acid (FA)</b> ameliorated D-galactose induced spatial learning and memory impairment, reduced exploratory ability through attenuating oxidative stress, inhibiting AChE activity and suppressing neuroinflammation and neurodegeneration.	(Yang et al., 2016)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
			was more effective than low dose FA (50mg/kg/d) in all parameters.			
10-week-old Kunming mice Groups: (1) control (0.9 % saline, s.c) (2) D-gal (50 mg/kg/d, s.c) (3) D-gal (50 mg/kg/d, s.c) + UA (10 mg/kg/d, p.o) (4) UA (10 mg/kg/d, p.o)  Duration: 8 weeks	- Step-through Test - Morris Water Maze Test - NF-κB, COX-2, iNOS, TNFα, IL-1β, IL-6 by Western Blot - AGEs, RAGE by Immunosorbent Assay - GFAP, CD11b by Immunofluorescence Staining - ROS, protein carbonyl by chromatography	Prefrontal cortex	- <b>Morris water maze:</b> • ↓ Escape latency • ↑ Number of crossing • ↑ Time spent in the target quadrant  - <b>Step-through passive avoidance task:</b> • ↓ Latency time	- <b>Oxidative stress:</b> • ↓ Protein Carbonyl • ↓ ROS  - <b>Inflammatory markers:</b> • ↓ COX-2, iNOS, TNFα, IL-1β, IL-6, NF-κB  - <b>Aging marker:</b> • ↓ AGEs, RAGE  - <b>Activated astrocytes &amp; microglia cells:</b> • ↓ GFAP, CD11b	<b>Ursolic acid</b> (UA) could attenuate D-gal-induced spatial learning and memory impairment via decreasing oxidative stress, inflammatory markers, aging marker, activation of astrocytes and microglial cells.	(Lu et al., 2010b)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
<p>2–3 month-old Male Swiss albino mice</p> <p>Groups:</p> <p>(1) Naive (0.5 % sodium carboxymethyl cellulose, 0.5 ml/100g/d, p.o)</p> <p>(2) D-Gal (100 mg/kg/d, s.c)</p> <p>(3) CAR (5 mg/kg/d, p.o)</p> <p>(4) CAR (2.5 mg/kg/d, p.o) + D-gal (100 mg/kg/d, s.c)</p> <p>(5) CAR (5 mg/kg/d, p.o) + D-gal (100 mg/kg/d, s.c)</p> <p>Duration: 6 weeks</p>	<p>- Spatial navigation task (Morris water maze task)</p> <p>- Maze acquisition phase (training)</p> <p>- Maze retention phase (testing for retention of the learned task)</p> <p>- Elevated plus maze paradigm</p> <p>- Assessment of gross behavioral activity</p> <p>- glutathione, catalase, superoxide dismutase, and glutathione-S-transferase activity, MDA, and nitrite level, AchE activity by spectrophotometer</p>	<p>Whole Brain (excluding cerebellum)</p>	<p><b>- Morris water maze:</b></p> <ul style="list-style-type: none"> <li>• ↔ initial acquisition latency</li> <li>• ↓ first retention latency</li> <li>• ↓ second retention latency</li> </ul> <p><b>- Elevated plus maze paradigm</b></p> <ul style="list-style-type: none"> <li>• ↔ initial transfer latency</li> <li>• ↓ first retention transfer latency</li> <li>• ↓ second retention transfer latency</li> </ul>	<p><b>-Antioxidant enzymes:</b></p> <ul style="list-style-type: none"> <li>• ↑ glutathione, catalase, superoxide dismutase, and glutathione-S-transferase activity</li> </ul> <p><b>-Oxidative stress:</b></p> <ul style="list-style-type: none"> <li>• ↓ MDA, and nitrite level</li> </ul> <p><b>- Neuromodulation:</b></p> <ul style="list-style-type: none"> <li>• ↓ AchE</li> </ul>	<p><b>Carvidilol (CAR)</b> attenuated D-galactose-induced spatial learning and memory impairment, oxidative stress and apoptosis by increasing antioxidant enzymes, oxidative phosphorylation, and cell viability.</p>	<p>(Kumar et al., 2009)</p>

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
			- ↔ locomotor activity			
			- Note: High dose CAR (5mg/kg/d) was more effective than low dose CAR (2.5mg/kg/d) in all parameters.			
3-month-old Male Laca mice Groups: (1) Naïve (0.5 % sodium carboxymethyl cellulose, 1 ml/100 g/d, p.o) (2) D-gal (100 mg/kg/d, s.c) (3) PIO (10 mg/kg/d, p.o) (4) PIO (30 mg/kg/d, p.o) (5) PIO (10	- Morris water maze task - Maze acquisition phase (training) - Maze retention phase (retention of the learned task) - glutathione, catalase, superoxide dismutase, and glutathione-S-transferase activity, MDA, and nitrite level, caspase3 by spectrophotometer	Whole Brain (excluding cerebellum)	- <b>Morris water maze:</b> • ↔ initial acquisition latency • ↓ first retention latency • ↓ second retention latency  - ↔ locomotor activity  - <b>BADGE</b>	- <b>Antioxidant enz:</b> • ↑ glutathione, catalase, superoxide dismutase, and glutathione-S-transferase activity  - <b>Oxidative stress:</b> • ↓ MDA,nitrite level  - <b>Apoptosis:</b> • ↓ caspase-3	<b>Pioglitazone (PIO)</b> attenuated D-galactose-induced spatial learning and memory impairment, oxidative stress and apoptosis by increasing antioxidant enzymes, oxidative phosphorylation, and cell viability in senescence mice through activation of PPAR $\gamma$ receptors.	(Prakash and Kumar, 2013)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
mg/kg/d, p.o) + D-gal (100 mg/kg/d, s.c) (6) PIO (30 mg/kg/d, p.o) + D-gal (100 mg/kg/d, s.c) (7) bisphenol A diglycidyl ether (PPAR <sub>γ</sub> antagonist) (15 mg/kg/d, p.o) + PIO (30 mg/kg/d, p.o) +D-gal (100 mg/kg/d, s.c) Duration: 6 weeks			<b>treatment</b> blocked the protective effect of pioglitazone.  - Note: High dose PIO (30mg/kg/d) was more effective than low dose PIO (10mg/kg/d) in all parameters except in caspase 3 activity.			
3-month-old Kumming mice Groups: (1) control (0.9 % saline, s.c) (2) D-gal (180 mg/kg/d, s.c)	- Water-maze test - Step-down test - ROS by spectroflurometer - AGE by ELISA - MDA, SOD, GPx, CAT, T-AOC by spectrophotometer - T-ChE by commercial	Whole Brain	- <b>Water maze test:</b> • ↓ Escape latency • ↑ Number of touching the blind  - <b>Step-down test:</b>	- <b>Oxidative stress:</b> • ↓ MDA  - <b>Inflammatory markers:</b> • ↓ NF-KB, TNF-α & IL-6  - <b>Antioxidant enz:</b>	<b>FGF21</b> protected the aging mice brain from D- gal-induced spatial learning and memory impairment by attenuating oxidative stress, inflammatory markers and renewing the activities of antioxidant enzymes and	(Yu et al., 2015)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
(3) D-gal (180 mg/kg/d, s.c) + FGF21 (1 mg/kg/d, s.c) (4) D-gal (180 mg/kg/d, s.c) + FGF21 (2 mg/kg/d, s.c) (5) D-gal (180 mg/kg/d, s.c) + FGF21 (5 mg/kg/d, inj) 6. FGF21 (5 mg/kg/d, s.c)  Duration: 8weeks	kit - AR, SDH, RAGE, TNF- $\alpha$ , IL-6 by PCR - NF-KB by Western Blot		<ul style="list-style-type: none"> <li>• ↓ number of errors</li> <li>• ↑ step down latency</li> </ul> <p>-Note: All doses of FGF21 show the same effects.</p>	<ul style="list-style-type: none"> <li>• ↑ SOD, GPx, CAT, T-AOC</li> </ul> <p><b>- Aging marker:</b></p> <ul style="list-style-type: none"> <li>• ↓ AGE, AR, SDH, RAGE</li> </ul> <p><b>- Neuromodulation:</b></p> <ul style="list-style-type: none"> <li>• ↓ T-chE</li> </ul>	decreasing AGE formation, TChE activity.	
3- month-old Kunming mice Groups: (1) Control (2) D-gal (200 mg/kg/d, i.p) (3) D-gal (200 mg/kg/d, i.p) +	- Morris water Maze test - p21, p53, A $\beta$ <sub>1-42</sub> by Western Blot - AGEs, TNF- $\alpha$ , IL-6, SOD, GSH-Px, MDA, CAT, T-AOC, NO and NOS by commercial kits - TNF- $\alpha$ , IL-6, AGEs by	Hippocampus	<p><b>- Morris water maze:</b></p> <ul style="list-style-type: none"> <li>• ↓ Escape latency</li> <li>• ↑ Number of crossing</li> <li>• ↑ Time spent in the target</li> </ul>	<p><b>-Oxidative stress:</b></p> <ul style="list-style-type: none"> <li>• ↓ MDA</li> <li>• ↓ NO levels</li> <li>• ↓ NOS</li> </ul> <p><b>- Antioxidant enz:</b></p> <ul style="list-style-type: none"> <li>• ↑ GSH-Px, SOD, CAT,</li> </ul>	<b>Hyperbaric treatment (HBOT)</b> can prevent cognitive impairment and hippocampal senescence by retaining the hippocampal BDNF expression, anti-oxidation, anti-inflammation and	(Chen et al., 2016)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
Vit E (0.2 g/kg/d, i.g) (4) D-gal (200 mg/kg/d, i.p) + HBOT (0.25 MPa at a rate of 100 kPa/min)  Duration: 8 weeks	ELISA - p16, p21, p53 by RT-PCR - immunoreactive cells for BDNF by Immunohistochemical analysis		quadrant	TAOC  - <b>Inflammatory markers:</b> • ↓ TNF- $\alpha$ , IL-6  - <b>Aging marker:</b> • ↓ AGE • ↓ p16, p21, p53 • ↓ A $\beta$ <sub>1-42</sub>  - ↑ BDNF positive cells - ↑ surviving neurones	modulation of aging-related gene expression in a mouse model of D-galactose-induced aging.	
Adult Male BALB/c mice Groups: (1) control (0.9 % saline, s.c) (2) D-gal +sham (500	- Active mitochondria levels by MitoTracker Green staining - Mitochondrial membrane potential determination by Mitochondria Staining Kit	Whole brain	- <b>Barnes Maze test</b> • ↓Escape latency • ↓Time spent in error holes • ↑Time spent in the target	- <b>Oxidative stress:</b> • ↓ ROS  - <b>Apoptosis:</b> • ↓ Bax/Bcl-2 ratio • ↓ Caspase-3	<b>Transcranial low level laser therapy (LLLT)</b> abrogated oxidative stress, apoptosis leading to reverse cognitive impairment in D-galactose-induced aging mice.	(Salehpour et al., 2017)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
mg/kg/d, s.c) (3) D-gal + red 4 (D-gal +4 J/cm <sup>2</sup> of red laser) (4) D-gal+ NIR 4 (D-gal+ 4 J/cm <sup>2</sup> of NIR laser) (5) D-gal+ red 8 (D-gal+ 8 J/cm <sup>2</sup> of red laser) (6) D-gal+ NIR 8 (D-gal+ 8 J/cm <sup>2</sup> of NIR laser)  Duration: 6 weeks	- Mitochondrial cytochrome c oxidase activity by commercial kits - Bax/Bcl-2, Caspase 3 by western blot - ATP levels by Colorimetric assay kit - ROS by Fluorescent dye dichlorohydrofluorescein diacetate - Barnes Maze test - What-Where-Which task		quadrant • ↑Relative error time  - <b>What-Where- Which task</b> • ↔Locomotor activity • ↔Total observation time • ↑Displacement index	- Note: Higher dose of LLLT exerted a better response than low dose (4 J/cm <sup>2</sup> ).		
3-month-old	- Morris water maze test	Hippocampus	- <b>Morris water</b>	- <b>Inflammatory</b>	<b>Salidroside</b> (sal)	(Gao et

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
Male Sprague-Dawley rats Groups: (1) control (0.9 % saline, s.c) (2) D-gal (120 mg/kg/d, s.c) (3) D-gal (120 mg/kg/d, s.c) + sal treatment (20 mg/kg/d, p.o) (4) D-gal (120 mg/kg/d, s.c) + sal treatment (40 mg/kg/d, p.o).  Duration: 6 weeks	- Step-down type passive avoidance test  - IL-1 $\beta$ , TNF- $\alpha$ , IL-6 by ELISA  - Txnip, p-NF- $\kappa$ Bp65, p-I $\kappa$ B $\alpha$ , p-IKK $\alpha$ , p-IKK $\beta$ , Bax/Bcl-2 ratio, caspase-9 by Western blot		<b>maze:</b> <ul style="list-style-type: none"> <li>• ↓ Escape latency</li> <li>• ↑ Number of crossing</li> <li>• ↑ Time spent in the target quadrant</li> </ul> <b>- Step-down test:</b> <ul style="list-style-type: none"> <li>• ↓ number of errors</li> <li>• ↑ step down latency</li> </ul> -Note: All doses of sal show the same effects.	<b>markers:</b> <ul style="list-style-type: none"> <li>• ↓ TNF-<math>\alpha</math>, IL-6, IL-1<math>\beta</math></li> <li>• ↑ Txnip</li> <li>• ↓ p-NF-<math>\kappa</math>Bp65, p-I<math>\kappa</math>B<math>\alpha</math>, p-IKK<math>\alpha</math>, p-IKK<math>\beta</math></li> </ul> <b>- Apoptosis:</b> <ul style="list-style-type: none"> <li>• ↓ Bax/Bcl-2 ratio</li> <li>• ↓ Caspase-9</li> </ul>	prevented D-gal-induced spatial learning and memory impairment by the effect of anti-inflammatory and anti-apoptotic responses in the hippocampus.	al., 2015)
3-month-old Sprague-Dawley rats Groups: (1) control	- Morris Water Maze Test - GSH-px activity and GSH content, SOD activity and MDA by	Hippocampus	<b>- Morris water maze:</b> <ul style="list-style-type: none"> <li>• ↓ Escape latency</li> <li>• ↑ Number of</li> </ul>	<b>-Oxidative stress:</b> <ul style="list-style-type: none"> <li>• ↓ MDA</li> </ul> <b>- Antioxidant enz:</b> <ul style="list-style-type: none"> <li>• ↑ GSH, SOD</li> </ul>	<b>Ginsenoside (Rg1)</b> treatment can improve D-galactose-induced spatial learning and memory impairment and hippocampus senescence	(Zhu et al., 2014)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
(0.9% saline, s.c) (2) D-gal (120 mg/kg/d, s.c) (3) Rg1 (20 mg/kg/d, i.p) (4) D-gal (120 mg/kg/d, s.c) (5) D-gal + Rg1 (20 mg/kg/d, i.p)  Duration: 6 weeks	spectrophotometer - Detection of proinflammatory cytokines by ELISA - SA- $\beta$ -gal (Senescence associated $\beta$ - galactosidase cytochemical staining) - SOX2 by Western blot - telomere length by Southern blot - telomerase by TRAP-PCR - p53, p19 <sup>Arf</sup> , p21 <sup>Cip1/Waf1</sup> by qRT-PCR		crossing • $\uparrow$ Time spent in the target quadrant	<b>- Inflammatory markers:</b> • $\downarrow$ TNF- $\alpha$ , IL-6, IL-1 $\beta$  <b>- Hippocampal neurogenesis:</b> • $\leftrightarrow$ SOX2 • $\uparrow$ BrdU cells  <b>- Senescence-associated genes:</b> • $\downarrow$ p53, p19 <sup>Arf</sup> , p21 <sup>Cip1/Waf1</sup>  <b>- Function of the lysosomes:</b> • $\downarrow$ SA- $\beta$ -gal staining  <b>- Neuronal marker:</b> • $\uparrow$ $\beta$ -tubulin III  <b>- Activated astrocytes:</b> • $\downarrow$ Gal-c and	by involving in the anti-oxidation and antiinflammation regulating telomere length, hippocampus neurogenesis, senescence-associated genes, and function of lysosomes, neuronal marker and activation of astrocytes.	

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
				<p>GFAP</p> <ul style="list-style-type: none"> <li>• ↓ Nestin and Aeg1 astrocyte elevated gene-1</li> </ul> <p>- ↑ telomere lengths, telomerase activity</p>		
<p>3-month-old Sprague-Dawley rats</p> <p>Groups:</p> <p>(1) control (0.9 % saline, s.c)</p> <p>(2) D-gal (100 mg/kg/d, i.p.)</p> <p>(3) Anth (100 mg/kg/d, i.p)</p> <p>(4) D-gal (100 mg/kg/d, I.P) + Anth (100 mg/kg/d, i.p)</p> <p>Duration: 7 weeks</p>	<p>- Morris Water Maze Test</p> <p>- Y-Maze Test</p> <p>- Aβ, BACE-1, RAGE, TNFα, NF-κb, iNOS, Bax, Bcl-2, PARP-1, synaptophysin, syntaxin, SNAP-23, p-CREB by Western Blot</p> <p>- ROS by spectrofluorometer</p> <p>- MDA by fluorometric assay kit</p> <p>- 8-OxoG, p-JNK, GFAP, Iba-1, Aβ by Immunofluorescence Analysis</p>	Whole Brain	<p>- <b>Morris water maze:</b></p> <ul style="list-style-type: none"> <li>• ↓ Escape latency</li> <li>• ↑ Number of crossing</li> <li>• ↑ Time spent in the target quadrant</li> <li>• ↑ Swimming speed</li> </ul> <p>- <b>Y-Maze Test</b></p> <p>↑ The percentage of spontaneous</p>	<p>- <b>Oxidative stress:</b></p> <ul style="list-style-type: none"> <li>• ↓ MDA</li> <li>• ↓ 8-OxoG</li> <li>• ↓ ROS</li> </ul> <p>- <b>Inflammatory markers:</b></p> <ul style="list-style-type: none"> <li>• ↓ iNOS, TNFα, NF-κB</li> </ul> <p>- <b>Apoptosis:</b></p> <ul style="list-style-type: none"> <li>• ↓ Bax/Bcl-2</li> <li>• ↓ p-JNK</li> <li>• ↓ PARP-1</li> </ul> <p>- <b>Synaptic proteins:</b></p>	<p><b>Anthocyanins (Anth)</b> effectively attenuate D-galactose induced spatial learning and memory impairment, oxidative stress, neuroinflammation and reverse synaptic dysfunction, reducing amyloid protein and activation of astrocytes, microglia cells.</p>	(Rehman et al., 2017)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
			alteration	<ul style="list-style-type: none"> <li>• ↑ synaptophysin, syntaxin, SNAP-23, p-CREB</li> <li>- <b>Amyloid protein:</b> <ul style="list-style-type: none"> <li>• ↓ BACE-1, A<math>\beta</math></li> </ul> </li> <li>- <b>Aging marker:</b> <ul style="list-style-type: none"> <li>• ↓ RAGE</li> </ul> </li> <li>- <b>Activated astrocytes &amp; microglia cells:</b> <ul style="list-style-type: none"> <li>• ↓ GFAP, Iba-1</li> </ul> </li> </ul>		
4-month-old Wistar rats Groups: (1) control (methyl cellulose, 2 %, in distilled water, p.o) (2) D-gal (150	- Morris water maze task - GSH, GPx, MDA, protein thiol (-SH) groups, AOPP (Advanced oxidation protein products) by spectrophotometer -caspase 3 by Electrophoresis and	Whole Brain	- <b>Morris water maze:</b> <ul style="list-style-type: none"> <li>• ↑ Number of crossing</li> <li>• ↑ Time spent in the target quadrant</li> </ul>	- <b>Antioxidant enz:</b> <ul style="list-style-type: none"> <li>• ↑ GSH, GPx</li> </ul> - <b>Oxidative stress:</b> <ul style="list-style-type: none"> <li>• ↓ MDA, AOPP and protein carbonyls</li> </ul> - <b>Apoptosis:</b>	<b>Curcumin and hesperidin (HES)</b> were found to improve D-galactose induced spatial learning and memory impairment and the functional capacity of neurons, reducing the probability of apoptosis	(Banji et al., 2014)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
mg/kg/d, s.c) (3) Curcumin (50 mg/kg/d, p.o) + D-gal (150 mg/kg/d, s.c) (4) HES (10 mg/kg/d) + D- gal (150 mg/kg/d, s.c) (5) Curcumin (50 mg/kg/d) + HES (10 mg/kg/d) + D- gal (150 mg/kg/d, s.c) (6) Curcumin (100 mg/kg/d) + HES (25 mg/kg) + D- gal (150 mg/kg/d, s.c)  Duration: 9 weeks	western blot		- Note: Higher dose of the combination of curcumin with HES exerted a better response than low dose combination and individual therapy. •	• ↓ caspase-3  - improved architecture of neurons in CA1 region	and oxidative stress.	
Adult Sprague Dawley rats	- Y-maze task	Hippocampus,	- <b>Y-Maze Test</b>	- <b>Synaptic</b>	<b>Caffeine</b> attenuated D-	(Ullah et

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
Groups: (1) 0.9 % saline control (2) D-gal (120 mg/kg/d, i.p) (3) D-gal (120 mg/kg/d, i.p) + Caffeine (3 mg/kg/d, i.p) (4) Caffeine (3 mg/kg/d, i.p) Duration:60 D	- PJNK, COX-2, NOS 2, IL-1 $\beta$ , TNF- $\alpha$ , Cyt.C, PARP-1, Bax/Bcl-2, Caspase3, Caspase9, synaptophysin and PSD95 by Western Blot - PJNK, Caspase3, 8-oxoguanine by immunofluorescence analysis - Degenerating neurons by Fluoro-Jade B staining - Survival neurons by Cresyl violet staining	Cortex	↓ The percentage of spontaneous alteration	<b>proteins:</b> • ↑ synaptophysin, PSD95 <b>- Oxidative stress:</b> • ↓ 8-oxoguanine <b>- Inflammatory markers:</b> • ↓ COX-2, NOS-2, TNF $\alpha$ & IL-1 $\beta$ <b>- Apoptosis:</b> • ↑ anti-apoptotic Bcl2 • ↓ Bax • ↓Caspase-9, Caspase-3 • ↓ PARP-1 • ↓ p-JNK <b>- Degenerating neurons:</b> • ↓ FJB + neuronal cells	galactose induced spatial learning and memory impairment, synaptic dysfunction, oxidative stress, apoptosis, neuroinflammation and neurodegeneration.	al., 2015)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
Adult Male Wistar rats Groups: (1) Control group (0.9 % saline, s.c) (2) D-gal (100 mg/kg/d, s.c) (3) D-gal (100 mg/kg/d, s.c) + THP (20 mg/kg/d, p.o) (4) D-gal (100 mg/kg/d, s.c) + THP (40 mg/kg/d, p.o) (5) D-gal (100 mg/kg/d, s.c) + THP (80 mg/kg/d, p.o)  Duration: 8 weeks.	- Morris water maze test - Open field test - MDA, NO, GSH, ACh, GPx, SOD, CAT, AChE by commercial kit - NF-κB, caspase-3, GFAP by Immunohistochemical analysis - Histopathological analysis	Whole Brain	- <b>Morris water maze:</b> • ↓ Escape latency • ↑ Number of crossing • ↑ Time spent in the target quadrant  - <b>Open field test:</b> • ↑ Number of grid crossing • ↑ Number of rearing and learning  -Note: All doses of THP show the same effects.	- <b>Survival neurons:</b> ↑ Cresyl violet neurones  - <b>Oxidative stress:</b> • ↓ MDA • ↓ NO levels  - <b>Antioxidant enz:</b> • ↑ GSH, SOD, CAT and GPx - <b>Neuromodulation:</b> • ↓ AChE • ↑ ACh  - <b>Apoptosis:</b> • ↓ Caspase-3  - <b>Inflammatory markers:</b> • ↓ NF-κB  - improved damaged neurons in the hippocampus CA1 region	<b>Tetrahydropalmatine (THP)</b> attenuated D-galactose induced spatial learning and memory and locomotor and behavioral activity impairment, neurochemical deficits through decreasing oxidative damage, dysfunction of the cholinergic system, inflammatory markers and activation of astrocytes.	(Qu et al., 2016)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
Middle-aged Sprague-Dawley rats Groups: (1) control (0.9 % saline, s.c) (2) Mimetic aging group (10 % D-gal, 1 ml/kg/d, s.c) (3) DHEA-treated normal group (1 ml/kg/d, i.p) (4) Vehicle control group (2 % DMSO, i.p + 10 % D-gal, 1 ml/kg/d, s.c ) (5) 2 % DHEA-treated senescent group (1 ml/kg/d, i.p) +	- Morris water maze test - Peripheral-type benzodiazepine binding sites by autoradiographic localization	Cerebral cortex	- <b>Morris water maze:</b> • ↓ Escape latency • ↑ Time spent in the target quadrant • ↑ Swimming distance	- <b>Antioxidant ability:</b> • ↑ mitochondrial peripheral benzodiazepine receptors	<b>Dehydroepiandrosterone (DHEA)</b> could improve spatial learning and memory in d-galactose-induced senescent rats, perhaps due to its ability to physiologically strengthen the antioxidant ability in mitochondria.	(Chen et al., 2008)

Study Model	Methods	Brain Area	Major finding		Interpretation	Ref
			Cognitive function	Related findings		
10 % D -gal, 1 ml/kg/d, s.c						
Duration: 8 weeks						

s.c: subcutaneous; p.o: per oral; NADH: Nicotinamide adenine dinucleotide; MDA: malondialdehyde; NO: nitric oxide; SOD: superoxide dismutase; AchE: acetyl choline esterase; IL: interleukin; NF- $\kappa$ B: nuclear factor kappa-light-chain-enhancer of activated B cells; ELISA: enzyme-linked immunosorbent assay; COX: Cyclooxygenase; TNF: tumor necrosis factor alpha; GFAP: Glial fibrillary acidic protein; ROS: reactive oxygen species; TNOS: total nitric oxide synthase; iNOS: inducible nitric oxide synthase; 8-OHdG: 8-hydroxy-2'-deoxyguanosine; Ach E: acetylcholinesterase; NOX: NADPH oxidase; p22<sup>phox</sup>, p47<sup>phox</sup>, p67<sup>phox</sup>: subunit of NOX; cyt c: cytochrome c; bp: base pair; PCR: polymerase chain reaction; TRAP-PCR: Telomeric repeat amplification protocol; GSH: glutathione; GPx: glutathione peroxidase; CAT: catalase; T-AOC: total antioxidant capacity; AGEs: advanced glycation endproducts; RAGE: AGE receptors; PCR: polymerase chain reaction; SA- $\beta$ -gal: senescence-associated  $\beta$ -galactosidase; Txnip: thioredoxin-interacting protein; p-IKK $\alpha$ , p-IKK $\beta$  phosphorylated I $\kappa$ B kinase  $\alpha$ ,  $\beta$ ; Bcl: B-cell lymphoma; Bax: bcl-2-like protein 4; SOX: SRY (sex determining region Y)-box 2; p53, p19<sup>Arf</sup>, p21<sup>Cip1/Waf1</sup>: senescence-associated genes; BrdU: Bromodeoxyuridine; Gal-c: galactosylceramidase; Aeg1: astrocyte elevated gene-1; A $\beta$ : amyloid beta protein; BACE-1:  $\beta$ -site amyloid precursor protein cleaving enzyme 1; PARP: poly (ADP-ribose) polymerase; SNAP-23: synaptosomal-associated protein 23; p-CREB: cAMP response elements binding; p-JNK: phosphorylated c-Jun N-terminal kinases; Iba-1: ionized calcium-binding adapter molecule 1; PSD95: postsynaptic density-95; FGF21: fibroblast growth factor 21; BDNF: Brain-derived neurotrophic factor

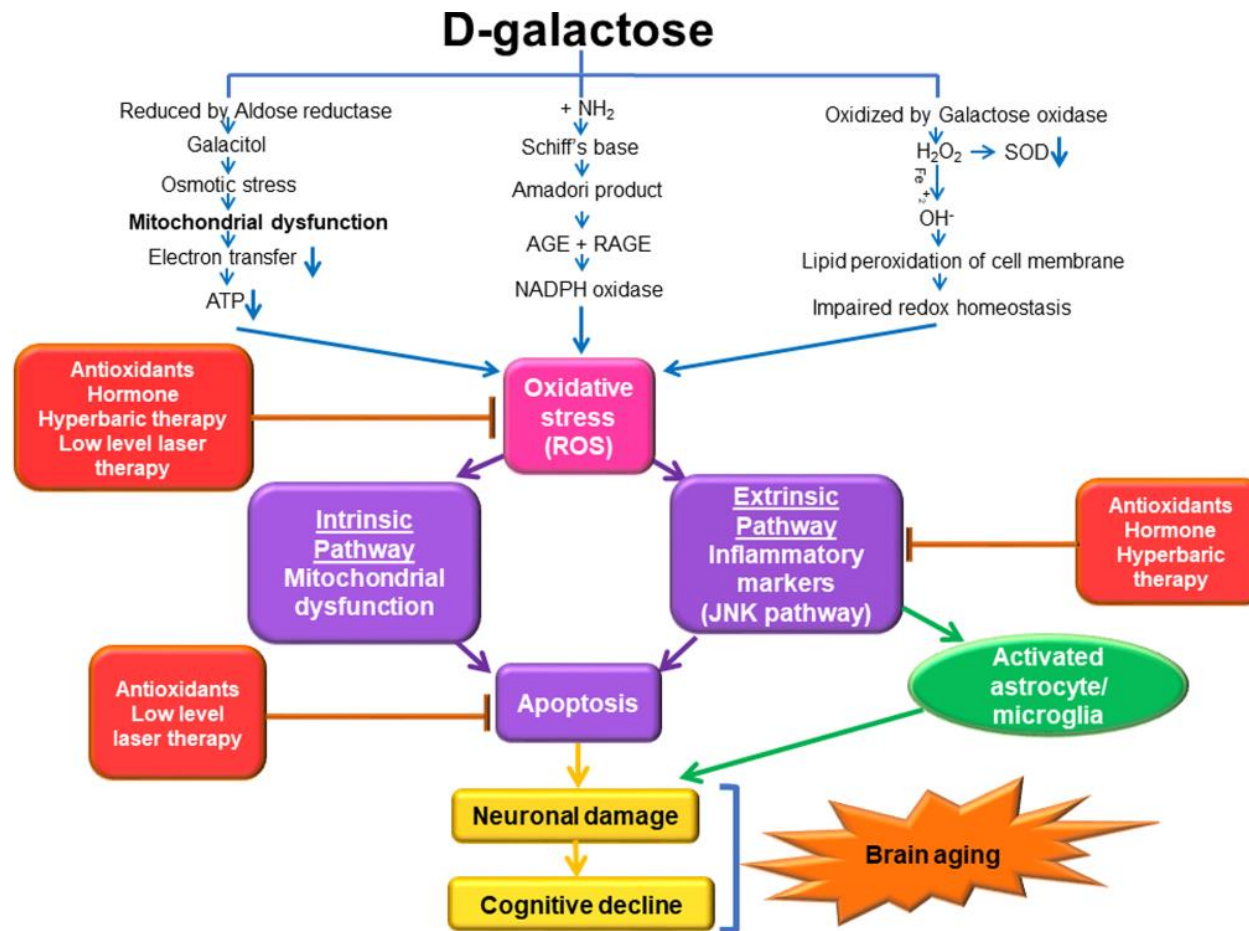


Figure 1

**Highlights**

- D-galactose is the artificial senescence drug to induce aging process.
- D-galactose induces brain aging via mitochondrial dysfunction.
- Various interventions could reverse D-galactose induced brain aging.